

*Epithelial Integrity as a Tumor Suppressor Mechanism –
The Interplay Between Lkb1 and c-Myc in Breast Cancer
Development*

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ACADEMIC DISSERTATION

*To be publicly discussed, with the permission of Faculty of Medicine,
University of Helsinki, in Biomedicum Helsinki, Lecture hall 2, on June 7th at
12 o'clock noon.*

Helsinki 2012

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ISBN 978-952-10-8006-7 (paperback)
ISBN 978-952-10-8007-4 (PDF)
<http://ethesis.helsinki.fi>
Unigrafia 2012

*“Tis strange – but true
for truth is always strange;
stranger than fiction”*

Lord Byron

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ABBREVIATIONS

AJ	Adherens junction
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
aPKC	Atypical protein kinase C
ARF	Alternative reading frame
ARK	AMPK-related kinase
ATM	Ataxia-telangiectasia mutated
ATP	Adenosine triphosphate
bHLHZip	Basic helix-loop-helix-leucine zipper
BM	Basement membrane
BRCA1/2	Breast cancer 1/2
BRSK	Brain-specific kinase
Cdc42	Cell and division cycle 42
CDK	Cyclin dependent kinase
CIP2A	Cancerous inhibitor of PP2A
CRB	Crumbs polarity complex
CRIB	Cdc42 and Rac interactive domain
DCIS	Ductal carcinoma in situ
DISC	Death inducing signaling complex
ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial-to-mesenchymal transition
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinase
FAK	Focal adhesion kinase
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FOXO3a	Forkhead box 3a
GH	Growth hormone
GSK3 β	Glycogen synthase kinase 3 β
GTP	Guanine triphosphate
HGF	Hepatocyte growth factor
IGF	Insulin-like growth factor
ILK	Integrin-linked kinase
K-Ras	Kirsten rat sarcoma viral oncogene homolog
LGL	Lethal giant larvae
LKB1/STK11	Liver kinase B1/Serine-threonine kinase 11
LOH	Loss of heterozygosity
MAP	Microtubule-associated protein
MARK	Microtubule affinity regulated kinase
MB	Myc box
MDCK	Madin-Darby canine kidney cell
Mdm2	Mouse double-minute 2
MEC	Mammary epithelial cell

MMP	Matrix metalloproteinase
MMTV	Mouse mammary tumor virus
mTOR	Mammalian target of rapamycin
MO25	Mouse protein 25
MOMP	Mitochondrial outer membrane permeability
MT-MMP	Membrane-type matrix metalloproteinase
MUPP	Multi PDZ-domain protein
NF-κB	Nuclear factor k-B
NSCLC	Non-small cell lung carcinoma
PALS1	Protein associated with lin 7 1
PAR	Partitioning defective
PATJ	PALS1-associated tight junction protein
PB1	Phox-Bem1p domain
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PDZ	PSD-95, Discs large, ZO-1 domain
PI3K	Phosphatidyl-inositol 3 kinase
PIP	Phosphatidyl-inositol
PJS	Peutz-Jeghers polyposis syndrome
PP2A	Protein phosphatase 2A
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog
ROCK	RhoA kinase
SCRIB	Scribble polarity complex
SIK	Salt-induced kinase
SNRK	SNF/AMPK related kinase
Stat5a	Signal transducer and activator of transcription 5a
STRAD	Ste20-related adaptor protein
TAD	Transactivation domain
TDLU	Terminal ductal lobular unit
TEB	Terminal end bud
TGF-β1	Transforming growth factor β1
TJ	Tight junction
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis inducing ligand
TSC1/2	Tuberous sclerosis 1/2
TSG	Tumor suppressor gene
uPA	urokinase plasminogen activator
WAP	Whey acidic protein
ZO	Zona occludens

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by their roman numerals.

- I **Partanen, J.I.**, Nieminen, A.I., Makela, T.P. and Klefstrom, J. (2007) Suppression of oncogenic properties of c-Myc by LKB1-controlled epithelial organization. *Proc Natl Acad Sci U S A*, 104, 14694-14699.

- II Nieminen, A. I., **Partanen, J.I.**, Hau, A., and Klefstrom, J. (2007). c-Myc primed mitochondria determine cellular sensitivity to TRAIL-induced apoptosis. *Embo J* 26, 1055-1067.

- III **Partanen, J.I.**, Tervonen T.A., Myllynen M., Lind E., Makela, T.P. and Klefstrom, J. (2012) Tumor suppressor function of Liver kinase B1 (Lkb1) is linked to regulation of epithelial integrity. *Proc Natl Acad Sci U S A*, 109, E388-397

- IV **Partanen, J.I.**, Tervonen, T.A., Marques, E., Myllynen, M., Lepikhova, T., Monni, O., Laakso, M., Hautaniemi, S. and Klefstrom, J. An shRNA-in silico approach identifies genes regulating epithelial integrity-dependent proliferation suppression. *Manuscript*.

ABSTRACT

Cancer progresses towards malignancy by gradual acquisition of genetic lesions that activate proto-oncogenes and inactivate tumor suppressor genes. Loss of cell polarity and cell-cell contacts are common in advanced solid cancers and often indicate aggressive disease and high metastatic potential of the primary tumor. However, the role of epithelial integrity, formed by the coordinated interplay between specialized cell adhesions and cell polarity, in the initiation of human cancers has been less clear.

The major aim of this study was to explore the role of epithelial integrity as a tumor suppressor mechanism in human and murine mammary epithelial cells. The mammary gland is a unique organ, where most of its development occurs post-natally and is affected by cyclical hormonal activity. Consequently, the mammary gland needs to accommodate vast amounts of cell proliferation and tissue remodeling without compromising proliferation control. Using a three-dimensional cell culture model recapitulating many aspects of the mammary epithelium *in vitro*, we observed that formation of intact epithelial integrity could protect cells from excess proliferation induced by the c-Myc oncoprotein. Furthermore, disruption of epithelial integrity by loss of the apico-basal polarity gene and tumor suppressor Lkb1, restored the ability of c-Myc to induce cell cycle progression. Correspondingly, loss of Lkb1 from mouse mammary epithelial cells *in vitro* and *in vivo* led to widespread loss of epithelial integrity, as demonstrated by loss of apico-basal polarity, basement membrane (BM) deterioration and increased side branching. Furthermore, while loss of Lkb1 alone did not induce tumor formation in the mouse mammary gland, it synergized with c-Myc leading to dramatically increased tumorigenesis. Interestingly, the synergy between Lkb1 loss and c-Myc was observed to involve deregulation of serine-protease Hepsin, a BM degrading protease also implicated in human cancers.

A second aim of this study was to study the mechanisms of c-Myc-induced sensitization to apoptosis and how it is modulated by epithelial integrity. We found that c-Myc induces apoptotic sensitivity specifically to TNF-related death inducing ligand (TRAIL) through activation of pro-apoptotic protein Bak and amplification of mitochondrial apoptotic signaling. Also, the apoptotic function of c-Myc was modulated by the epithelial integrity while mechanisms of c-Myc-induced apoptotic sensitivity was not. Finally, we also observed that shRNA-mediated downregulation of human orthologues of *Drosophila* epithelial integrity regulators causes disruption cell proliferation control leading to excess proliferation but interestingly, also to synthetic lethal phenotypes in combination with c-Myc activation. The findings presented in this thesis thus propose that the structural organization of epithelial cells as an intact epithelial integrity can modulate the action of single oncogenes, in this case c-Myc, and present an important barrier for tumor formation.

I REVIEW OF THE LITERATURE

1. MAMMARY GLAND

The mammary gland is a unique organ, as most of its development occurs post-natally and it undergoes continuous remodeling due to the cyclical reproductive hormones and pregnancies. The mammary epithelium starts to develop already before birth, but the full development of the mammary ductal tree starts only at puberty. The complete formation and differentiation of lobulo-alveolar structures is accomplished during pregnancy and lactation. After the cessation of lactation, the lobulo-alveolar structures involute and the epithelium reverts to the state prior to the pregnancy (Richert *et al.*, 2000). Thus, it is essential that the extensive growth, differentiation and involution cycles occurring during the reproductive phase be well controlled. The need for strict proliferation control is further underlined by the fact that the mammary gland is prone to development of epithelial cancers, suggesting that the cyclical changes and the vast amount of epithelial remodeling can act as a predisposing event in tumorigenesis. Consequently, the mammary gland is an organ where the epithelium has an important role in its normal function and also in disease.

1.1 Structure of the mammary gland

The mammary gland is an apocrine gland consisting of glandular epithelium surrounded by a microenvironment composed of extracellular matrix (ECM), adipocytes and diverse stromal cell types (Polyak and Kalluri, 2010; Richert *et al.*, 2000). The mammary epithelium forms a branched ductal network with ducts draining to an opening in the nipple. At the posterior end of each duct are situated terminal ductal lobular units (TDLU) (Figure 1A), which contain lobules consisting of several secretory alveoli (or acini). Alveoli are blunt ended units that differentiate during lactation to produce milk and thus perform the fundamental function of the mammary gland. Alveoli within each lobule are connected via collecting ducts to the main ducts, which during lactation drain the milk (Richert *et al.*, 2000).

The structure of the mammary epithelium is highly organized. Ducts and alveoli are composed of two layers of epithelial cells, an inner layer of polarized luminal epithelial cells and an outer layer of myoepithelial cells (Polyak and Kalluri, 2010) surrounded by a layer of basement membrane (BM) (Figure 1A). The inner luminal cell layer is responsible for the production of milk constituents and secretion into the lumen of the alveoli. The myoepithelial cell layer is essential in supporting the secretory function of the mammary gland, by being able to contract and aid the flow of milk from the alveoli to the lumen of the ducts. Additionally, it has been shown that the mammary stem cell function segregates with the basal lineage (Shackleton *et al.*, 2006; Sleeman *et al.*, 2006; Stingl *et al.*, 2006), and moreover the activated mammary stem cells are also found from the basal compartment of the mammary gland (Bai and

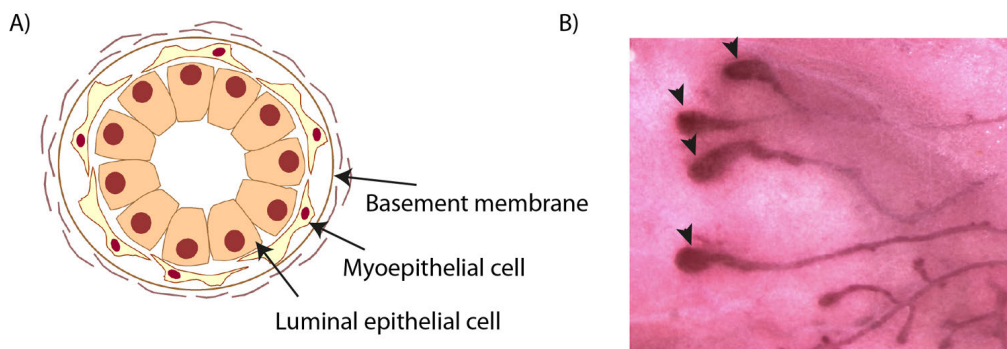


Figure 1. Structure of mammary epithelium and terminal end bud. A) A schematic illustration of the structure of mammary epithelium. The inner luminal epithelial cell layer, which is facing the lumen of the duct, is surrounded by contractile myoepithelial cells and the basement membrane. B) A carmine-alum stained mammary gland wholemount of an 8-week old mouse shows developing epithelial ducts, which terminate to a bulbous structure of terminal end bud (arrowheads).

Rohrschneider, 2010). Thus, whereas the luminal cells accomplish the differentiated function of the mammary gland, the basal epithelial cell layer appears to play a key role in the growth and renewal of the mammary gland.

There are known differences between human and mouse mammary glands, yet there are marked similarities in composition and function (Parmar and Cunha, 2004). Also, as several key studies elucidating the molecular biology of the mammary gland are performed in mice, the following chapters will concentrate on the development and differentiation of the murine mammary gland.

1.2 Mammary gland development - from embryo to adulthood

The development of the mammary gland starts during mouse embryonic development on embryonic day E10.5 by the formation of bilateral milk lines followed by formation of 5 pairs of mammary placodes within the milk line (Hens and Wysolmerski, 2005). Subsequently, between E11.5 and E12.5 cells invaginate from the ectoderm to the primary mammary mesenchyme to form an initial mammary bud. Following mammary bud formation, reciprocal interactions between the mammary bud and mammary mesenchyme originating from the mesoderm leads to formation of a rudimentary epithelial tree and condensation of the prospective mammary fat pad (Figure 2; Hennighausen and Robinson, 2001; Richert *et al.*, 2000). Indeed, signaling between the epithelium and mesenchyme has an important role during embryonic development of the mammary gland since at this stage the gland development is independent of systemic hormonal cues. For instance, disruption in parathyroid hormone-related protein (PTHrP), which is expressed both in the mesenchymal and epithelial compartments of the embryonic mouse mammary gland, leads to failure of the primary mammary mesenchyme to differentiate and subsequent failure of

mammary epithelium to develop past the mammary bud stage (Dunbar and Wysolmerski, 1999; Foley *et al.*, 2001; Wysolmerski *et al.*, 1998). Furthermore, signaling through Wnt, epidermal growth factor (EGF) and fibroblast growth factor (FGF) pathways appear to play key roles in the early development of the mammary gland (Hennighausen and Robinson, 2001; Hens and Wysolmerski, 2005). Notably, four of the five mammary placodes fail to form in the absence of FGF receptor 2b (FGFR2b) or its ligand FGF10 (Veltmaat *et al.*, 2003). Also, mammary glands of mice lacking Wnt pathway effector Lef1, or glands expressing Dkk1, a secreted Wnt inhibitor, show no mammary gland development beyond the bud stage (Boras-Granic *et al.*, 2006; Chu *et al.*, 2004).

Post natally, the rudimentary mammary epithelium stays relatively dormant until puberty (Figure 2). Hormone receptors are expressed in the mammary gland already before puberty (Haslam and Nummy, 1992; Hovey *et al.*, 2001), however the growth of mammary epithelium becomes hormone-dependent only during puberty. At the onset of puberty, the ductal tree starts to elongate and fill the mammary fat pad with a simple ductal network in response to hormonal activity and following estrous cycles, will result in side branching of the epithelium, adding further complexity to the ductal tree (Briskin and O'Malley, 2010; Richert *et al.*, 2000). The major systemic hormones responsible for adult mammary gland development are the pituitary hormones growth hormone (GH) and insulin like growth factor-1 (Igf1), and ovarian estrogen and progesterone (Daniel *et al.*, 1987; Feldman *et al.*, 1999; Lydon *et al.*, 1995). Estrogen, GH and Igf1, out of which the latter two are already present before estrogen, have been demonstrated to play a key role in mammary gland development as mice lacking either these hormones or their receptors exhibit disrupted mammary development after puberty (Daniel *et al.*, 1987; Feldman *et al.*, 1999; Gallego *et al.*, 2001). Also, mice lacking progesterone receptor (PR) show significant yet milder deficiencies in mammary gland development. Indeed, estrogen and progesterone appear to function in a sequential fashion in the development of the mammary gland, as estrogen receptor (ER) α $-/-$ mammary glands fail to develop beyond puberty while PR $-/-$ mammary glands start to develop, yet have defects in side branching and formation of alveoli (Briskin *et al.*, 1998; Feng *et al.*, 2007; Haslam, 1989; Mallepell *et al.*, 2006). Estrogen thus seems to be the major mitogenic stimulus during puberty and is required to initiate the first phase of ductal elongation, whereas progesterone is essential for formation of ductal side branches and alveologenesis. Additionally, there is evidence of interplay between hormonal cues and growth factor pathways. For example, estrogen is shown to elicit its mitogenic function via EGF family member amphiregulin (Ciarloni *et al.*, 2007; Haslam, 1989; Mallepell *et al.*, 2006), and progesterone via Cyclin D1 as well as by a paracrine mechanism involving tumor necrosis factor (TNF) family member, receptor activator of NF- κ B ligand (RANKL) (Beleut *et al.*, 2010).

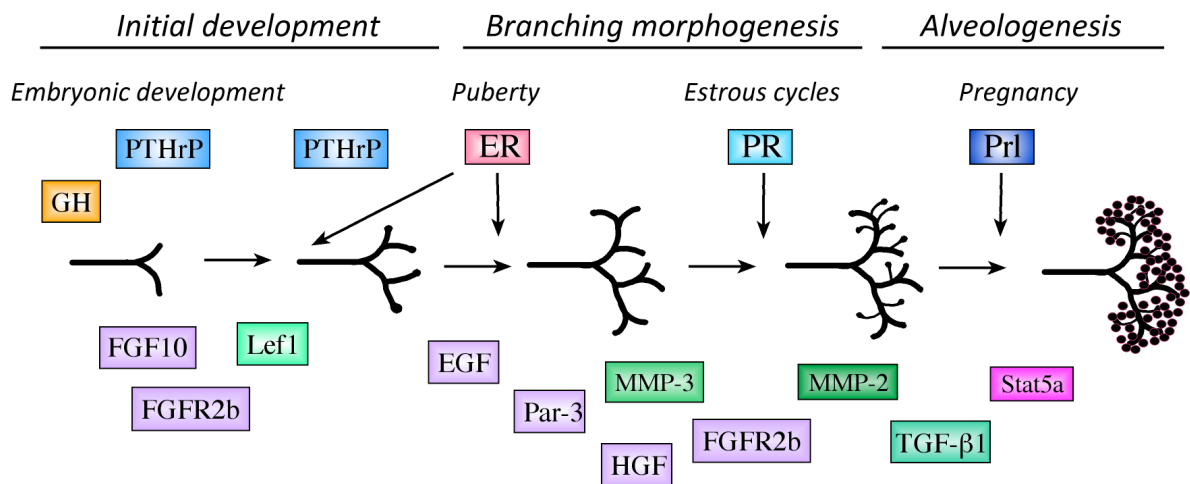


Figure 2. Mammary gland morphogenesis. A schematic representation of the different developmental phases and the changes occurring in the ductal epithelium during mammary gland morphogenesis. During embryonic development, growth of rudimentary mammary epithelium is observed and during the pre-pubertal phase, the growth continues to slowly form simple ducts. After the onset of puberty, the mammary epithelium starts to rapidly grow and undergo branching morphogenesis to form an elaborate ductal network. During pregnancy and lactation, the epithelium undergoes alveolar differentiation. Subsequently, after weaning the epithelium involutes and resumes its pre-pregnant state. Also shown are the major (GH, PTHrP, ER, PR, Prl on the upper side of the epithelium) and selected (eg. FGFR2b, Lef1, EGF, Par-3, Stat5a on the lower side of the epithelium) effectors of mammary gland morphogenesis and the specific phases where they function (Modified from Briskin & O'Malley 2010).

1.3 Branching morphogenesis

Branching morphogenesis is a process in which a single cell, or group of cells expands its area by forming extensions (Gray *et al.*, 2010; Lu and Werb, 2008) and thus increases the functional tissue area within an organ. Various organs such as the mammary gland, lungs, kidney, pancreas and salivary gland undergo branching morphogenesis as a part of their development (Gray *et al.*, 2010). In the mammary gland, branching morphogenesis can be divided into three phases: the embryonic phase, the pubertal phase and the adult phase. Embryonic branching leads to formation of a simple rudimentary epithelial tree, whereas the majority of branching events occur during the pubertal phase, when mammary gland development is expedited. During this growth phase, motile units termed terminal end buds (TEBs; Figure 1B), situated at the distal end of the elongating ducts, drive primary branching morphogenesis (Hinck and Silberstein, 2005). The TEBs are motile bulb-shaped units, which have a similar two-layered structure as the ducts they generate. The tip of the TEBs consists of outer cap cells, which invade the fat pad and inner body cells, forming the luminal structure (Hinck and Silberstein, 2005). The branch points form by

bifurcation of the TEB from one unit into two units (Gray *et al.*, 2010; Lu and Werb, 2008) and the TEBs undergo repeated branching until they reach the margins of the fat pad and regress (Richert *et al.*, 2000). Furthermore, unlike in other branching organs, in the case of mammary epithelium the branching process leaves open spaces between ducts to make room for lobulo-alveolar units to develop during pregnancy (Hinck and Silberstein, 2005). Additionally, simultaneous to the primary bifurcation occurs secondary branching laterally from the primary ducts that follow the TEBs (Sternlicht *et al.*, 2006). The adult branching phase, or tertiary branching, occurs as side branching to the existing ducts and is initiated during cyclical hormone stimulation and completes during pregnancy with lobulo-alveolar differentiation.

The branching process has been shown to require modifications of the surrounding ECM. It has been demonstrated that the ECM in front of and surrounding the invading TEB is rich in hyaluronic acid, while the ECM surrounding the mature ducts is rich in laminin, collagen IV and proteoglycans (Fata *et al.*, 2004; Lochter and Bissell, 1995; Silberstein and Daniel, 1982). Also, ECM remodeling enzymes have been shown to be indispensable for branching. For instance, overexpression of Stromelysin-1, a member of the matrix metalloprotease (MMPs) family, which is implicated in tissue invasion and branching morphogenesis of various tissues (Fata *et al.*, 2004), has been shown to lead to increased mammary duct side branching and disruption of the BM (Simpson *et al.*, 1994). Furthermore, while Stromelysin-1/MMP-3 was implicated in the formation of secondary and tertiary branches, MMP-2 was shown to play a role specifically in TEB invasion, duct elongation and in the inhibition of premature lateral branching (Wiseman *et al.*, 2003) suggesting that different MMPs act spatially and temporally differently to determine the pattern of branching. Also, some MMPs have been implicated in a more indirect role in branching morphogenesis, such as MMP-14, which seems to be required to diminish physical restraints of collagen I in the ECM during the branching process (Alcaraz *et al.*, 2011). Similarly, downregulation of tissue inhibitor of metalloprotease-1 (TIMP-1), a natural inhibitor of MMPs, leads to increased branching and degradation of BM component laminin (Fata *et al.*, 1999). Besides degradation of BM and ECM constituents, MMPs have been suggested to promote branching morphogenesis by cleaving of cell-cell adhesions, such as E-cadherin, and to induce transient mesenchymal characteristics in epithelial cells (Lochter *et al.*, 1997; Simian *et al.*, 2001). Also, various transmembrane ECM receptors, through which the epithelial cells sense their surroundings, have been implicated in the process. For example, it has been shown that deletion of integrin $\alpha 2$, but not $\alpha 3$ or $\alpha 6$, leads to reduction of branching (Chen *et al.*, 2002; Klinowska *et al.*, 2001). Similarly, deletion of $\beta 1$ -integrin exclusively from myoepithelial cells by Cre recombinase expressed under the K5 promoter or from the entire mammary epithelium by a function-blocking antibody, leads to defective branching (Klinowska *et al.*, 1999; Taddei *et al.*, 2008). Additionally, increased branching morphogenesis has been observed in mice lacking beta1,4-galactosyltransferase (Steffgen *et al.*, 2002), an ECM receptor binding to multiple ligands. Altogether, these data suggest that changes

in the remodeling and sensing of ECM as well as ECM composition are essential in the process of branching morphogenesis.

Numerous soluble growth factors and hormones have been linked to mammary branching morphogenesis. For instance, global regulators of mammary gland development, such as estrogen and progesterone, have been implicated in the process. Progesterone has been suggested to play an important role in this process via Wnt4 (Briskin *et al.*, 2000). Furthermore, prolactin has been shown to play a role in tertiary side branching of mammary epithelium (Hovey *et al.*, 2001; Ormandy *et al.*, 1997). Recently, FGFR2 and FGFR2b were both demonstrated to be needed for TEB functions and branching morphogenesis (Lu *et al.*, 2008; Parsa *et al.*, 2008). The importance of FGFs has further been emphasized by *in vitro* experiments, where isolated mammary organoids were induced to undergo branching morphogenesis in three-dimensional cell culture by the addition of FGF2 (Ewald *et al.*, 2008). Also, transforming growth factor β 1 (TGF- β 1) has been demonstrated to be a strong local inhibitor of branching morphogenesis and ductal elongation, as mice overexpressing TGF- β 1 show decreased amounts of TEB and compromised ductal development (Daniel *et al.*, 1989; Pierce *et al.*, 1993). Supporting these findings, increased amounts of TGF- β 1 were found in the stroma surrounding the mature ducts compared to the stroma surrounding TEBs (Silberstein *et al.*, 1992). On the other hand, it appears that some level of TGF- β 1 signaling is required for branching morphogenesis, as fewer TEBs develop in TGF- β 1-/- mammary glands (Ingman and Robertson, 2008). Correspondingly, overexpression of hepatocyte growth factor (HGF), which is negatively regulated by TGF- β 1, leads to increased lateral side branching and an increase in the amount of lateral end buds (Kamalati *et al.*, 1999). Comparably, conditional deletion of HGF receptor c-Met from the mouse mammary epithelial cells leads to defects in secondary ductal branching, but not in the bifurcation of TEBs (Garner *et al.*, 2011).

Finally, the complexity of the branching process is emphasized by the amount of factors also shown to regulate it (Summarized in Fata *et al.*, 2004; Lu and Werb, 2008). For instance, a recent study shows that loss of Par-3, a regulator of apico-basal cell polarity, leads to formation of abnormal TEBs and their inability to establish normal ducts (McCaffrey and Macara, 2009). Additionally, reduction in branching morphogenesis has been reported in mammary epithelium lacking Signal transducer and activator of transcription 5a (Stat5a) (Santos *et al.*, 2010; Vafaizadeh *et al.*, 2010), stem cell marker Prominin-1 (Anderson *et al.*, 2011), GATA-3, a member of a transcription factor family linked previously to cell fate specification in various tissues (Kouros-Mehr *et al.*, 2006), retinoic acid receptor α (Wang *et al.*, 2005) and primary cilia (McDermott *et al.*, 2010).

1.4 Pregnancy and lactation

The final developmental phase of the mammary gland occurs during pregnancy. In the first half of pregnancy, side branching is further increased and alveolar buds are formed (Richert *et al.*, 2000). During the second half of pregnancy, alveolar buds expand to give rise to lobulo-alveolar units, which further differentiate to secretory epithelium visible by enlargement of the epithelial cells and formation of proteinaceous secretion and large lipid droplets (Anderson *et al.*, 2007; Richert *et al.*, 2000). The secretory epithelium now fills up the majority of the mammary fat pad leaving only little space for stroma and adipocytes. At this stage, the myoepithelial cell layer encircling alveolar cells show discontinuities (Richert *et al.*, 2000) and therefore may allow for the alveolar cells to come into direct contact with BM, which has previously been demonstrated to be a critical step needed for full alveolar differentiation and milk production (Barcellos-Hoff *et al.*, 1989; Howlett and Bissell, 1993; Lee *et al.*, 1985; Streuli *et al.*, 1995).

After parturition and following the beginning of lactation, the alveolar cells start to secrete milk constituents to the alveolar lumen leading to visible flattening of the epithelial cells (Richert *et al.*, 2000). Myoepithelial cells aid the process of milk flow from the alveoli towards the nipple by contracting in response to oxytocin. Once started, suckling or milk removal from the ducts maintains the lactational state and the fully developed alveolar structures persist until the end of lactation (Anderson *et al.*, 2007; Neville *et al.*, 2002). The phase of alveolar differentiation and milk production is dependent on prolactin signaling, as mice lacking prolactin receptor show normal mammary ductal outgrowth but impaired alveologenesis and differentiation into secretory epithelium (Bole-Feysot *et al.*, 1998; Neville *et al.*, 2002; Ormandy *et al.*, 1997). Abrogation of prolactin signaling by deletion of its downstream target Jak2 has similarly been demonstrated to lead to impaired alveolar development (Wagner *et al.*, 2004). Furthermore, Stat5a, which in turn functions downstream of Jak2, has also been shown to be indispensable for alveolar development, possibly through its role in controlling the development of luminal progenitor cells (Liu *et al.*, 1997; Santos *et al.*, 2010; Yamaji *et al.*, 2009). Likewise, it was demonstrated recently that the alveolar differentiation could be induced by overexpression of Stat5a in the absence of ovarian hormones (Dong *et al.*, 2010). Also, other known downstream targets of prolactin such as Elf5 and SREBP1 have been implicated in alveolar differentiation (Harris *et al.*, 2006; Naylor *et al.*, 2005b).

Besides components of the prolactin signaling pathway, other genes have also been implicated in alveolar development and differentiation. Recently, luminally expressed GATA-3 transcription factor was shown to be required for full alveolar development and differentiation (Asselin-Labat *et al.*, 2007; Kouros-Mehr *et al.*, 2006). Interestingly, it has been shown that conditional knockout of β 1-integrin in luminal or myoepithelial cells, or its receptor fibronectin in luminal cells (Liu *et al.*, 2010; Naylor *et al.*, 2005a; Taddei *et al.*, 2008), leads to inability of epithelial cells to undergo alveolar

differentiation and lactation in response to prolactin. This further suggests that integrin-mediated adhesion of both luminal and myoepithelial cells to ECM, is required for full differentiation of mammary epithelium. In addition, correct timing of signaling appears to play a role in the onset of alveolar differentiation and thus also lactation. For example, overexpression of c-Myc in mammary epithelium interferes with the timing of alveolar differentiation, as c-Myc overexpression at a specific 72 hour time-window during mid-pregnancy leads to precocious alveolar development followed by premature apoptosis of alveolar cells and cessation of milk production (Blakely *et al.*, 2005). Similarly, overexpression of active TGF- β 1 or active TGF- β 1 receptor (T β RI) in mammary epithelium resulted in early apoptosis of differentiating alveolar cells and consequently inhibition of lactation (Jhappan *et al.*, 1993; Siegel *et al.*, 2003). Taken together, many of the same factors contributing to branching morphogenesis are also involved in alveolar differentiation.

After weaning, the stasis of milk in mammary ducts activates regression of the epithelium, termed involution. The secretory alveolar cells undergo apoptosis to revert the gland to the state before pregnancy. In addition to remodeling of the epithelium, the gland undergoes extensive influx of immune cells to remove remnants of apoptotic cells, as well as remodeling of the extracellular matrix and re-generation of adipocytes to fill the fat pad (Watson and Kreuzaler, 2011). The first, reversible phase of involution lasts approximately 48 hours in the mouse mammary gland. There are no clear morphological differences present at this stage, yet some apoptotic cells are seen (Richert *et al.*, 2000; Watson and Kreuzaler, 2011). The following, irreversible phase is characterized by apoptosis of alveolar cells leading to collapsed alveolar clusters and expansion of the stroma. The final stage of involution is orchestrated by the pro-apoptotic Bcl-2 family members and upregulation of the MMPs (Lund *et al.*, 1996; Metcalfe *et al.*, 1999) directing the mammary gland to resume to its pre-pregnant state (Richert *et al.*, 2000).

2. EPITHELIAL TISSUE STRUCTURE AND EPITHELIAL INTEGRITY

Epithelial tissue structure consists of physical adherence of epithelial cells to each other and their surroundings via specialized cell-cell and cell-ECM junctions (Reviewed by Bryant and Mostov, 2008). Additionally, epithelial tissue structure is sustained by cell polarity, the asymmetric distribution of macromolecules and intracellular organelles across the cell. Together, the adhesion and polarity of epithelial cells form a framework of epithelial integrity, which is essential for homeostasis and normal functions of epithelial tissues (Bryant and Mostov, 2008). Epithelial integrity thus directs the differentiation, signaling and dynamics of epithelial tissues as well as life and death decisions of individual epithelial cells.

2.1 Epithelial junctions

Epithelial cells are attached to one another via discrete cell-cell junctions with specialized functions (Figure 3). On the apical most side of epithelial cells reside tight junctions (TJs), or zonula occludens, an area where the cell membranes of two adjacent cells are closely associated. By sealing the apical side of the epithelium from the lumen, TJs function to sustain paracellular diffusion barrier and prevent diffusion of substances through the cell layer (Shin *et al.*, 2006). For instance, in the mammary gland TJs are of special importance in the secretory alveoli during lactation to seal the milk constituents in the lumen of the epithelium (Pitelka *et al.*, 1973). TJs are comprised of three types of transmembrane proteins: the junctional adhesion molecules (JAMs), claudins and occludins (Shin *et al.*, 2006). On the intracellular side, the TJ transmembrane proteins are connected by zonula occludens (ZO) -1, -2 and -3 scaffold proteins, which interact with claudins and occludins via their N-terminal Post synaptic density-95, Discs large, Zonula occludens-1 (PDZ) domains, and with the actin cytoskeleton via their C-terminal domain (Fanning *et al.*, 1998; Fanning *et al.*, 2002). Furthermore, the presence of both ZO-1 and -2 appears to be required for the formation of functional TJs, while it seems that ZO-3 is not essential for the process (Adachi *et al.*, 2006; Umeda *et al.*, 2006). Other proteins known to be intracellularly associated with TJs include non-PDZ protein cingulin, which has been shown to interact with other TJ proteins and is also implicated in the regulation of cell proliferation via RhoA inhibition (Aijaz *et al.*, 2005; Bazzoni *et al.*, 2000). Altogether, TJs contain some 40 proteins (Gonzalez-Mariscal *et al.*, 2003; Shin *et al.*, 2006), many of them intracellular TJ plaque proteins, therefore, TJs appear to be an important signaling platform for cell adhesion, growth and polarity signaling.

Below the apical region are found adherens junctions (AJs), or zonula adherens, initially characterized as electron dense plaques at cell-cell contacts (Figure 3). AJs mediate cell-cell adhesion via calcium dependent adhesion molecules, in epithelial cells E-cadherins, which contact E-cadherins of neighbouring cells by homotypic interactions. On the intracellular side E-cadherins bind α -, β - and γ -catenin, which in turn associate with other molecules and connect to the actin cytoskeleton (Adams *et al.*, 1998; Cavey and Lecuit, 2009; Drees *et al.*, 2005). In addition to cadherins, AJs also harbor the immunoglobulin like intermembrane proteins, nectins (Takahashi *et al.*, 1999). Nectins bind to intracellular afadin proteins, which have been demonstrated to bind α -catenin and actin and thus connect to cadherin adhesions and the cytoskeleton (Mandai *et al.*, 1997; Takahashi *et al.*, 1999). Hence, AJs consist of a relatively simple structure compared to TJs. However, they appear to be very dynamic structures and easily remodeled in developmental events such as epithelial-to-mesenchymal transition (EMT) and the converse MET. Furthermore, E-cadherin contacts are important landmarks for the establishment of cell polarity following MET: it has been proposed that they provide the initial cue for re-organization of the actin cytoskeleton and for polarity proteins to organize (Cavey and Lecuit, 2009).

Basally to AJs reside desmosomes, or macula adherens, which are adhesive cell junctions mediating stable adhesion. Transmembrane adhesion in desmosomes is mediated by desmosomal cadherins desmogleins and desmocollins, which are members of the cadherin superfamily (Kowalczyk *et al.*, 1994; Marcozzi *et al.*, 1998). Desmosomal cadherins show calcium-dependent adhesion, yet calcium-independent adhesion mechanisms have also been shown (Chitaev and Troyanovsky, 1997; Kimura *et al.*, 2007). The cytoplasmic tails of these proteins associate with desmosomal plaque proteins plakoglobin (γ -catenin), plakophilin and desmoplakin, which in turn connect the desmosomes to keratin intermediate filaments (Garrod and Chidgey, 2008). Desmosomes appear to be especially important in tissues requiring resistance to mechanical strength and needing strong adhesion, such as epidermis and cardiac myocytes. Moreover, both the desmosomal cadherins and plaque proteins are requisite for formation of this adhesion (Garrod and Chidgey, 2008). Interestingly, in the mammary gland desmosomes are rarely observed in the pregnant and lactating gland (Pitelka *et al.*, 1973), suggesting that more flexible adhesion is needed during alveolar differentiation. Additionally, it appears that desmosomes are not merely providing adhesion, but comparable to TJs, act as signaling platforms. For example, plakoglobin, a close relative of β -catenin, has been suggested to function as a negative regulator of β -catenin signaling (Miravet *et al.*, 2002; Zhurinsky *et al.*, 2000).

Gap junctions are small, pore-like junctions connecting the cytoplasm of two adjacent cells (Figure 3). They mediate intercellular communication by allowing an exchange of molecules between cells, and hence, gap junctions can have different functions depending on the tissue type (Plum *et al.*, 2000). For example, in the mammary gland gap junctions have been observed to form between luminal and myoepithelial cells during lactation and furthermore, gap junction proteins have been demonstrated to be important for production of milk proteins, secretion and lactation (El-Sabban *et al.*, 2003; Pitelka *et al.*, 1973; Plum *et al.*, 2000). Gap junctions are mainly formed by homo- or heterohexamers of connexin proteins, 14 of which are currently found in humans (Herve *et al.*, 2007; McLachlan *et al.*, 2007). Whether gap junctions are in open conformation, is thought to be regulated by phosphorylation of the connexins as well as by protein-protein interactions. Interestingly, connexins have been shown to bind a plethora of other junctional proteins including ZO-1, -2 and -3, occludin, claudin-1 and -5, p120 catenin and β -catenin (Herve *et al.*, 2007), suggesting that components of other junctions can control the function of gap junctions.

Epithelial cells interact with ECM and BM proteins via hemidesmosomes and various ECM receptors, such as integrins, dystroglycan and syndecan (Barresi and Campbel 2006, Morgan *et al.*, 2007). Hemidesmosomes resemble desmosomes structurally, as both are similarly connected to the intermediate filaments and transduce shear forces. Hemidesmosomes, however, provide an anchoring point for the cells into the

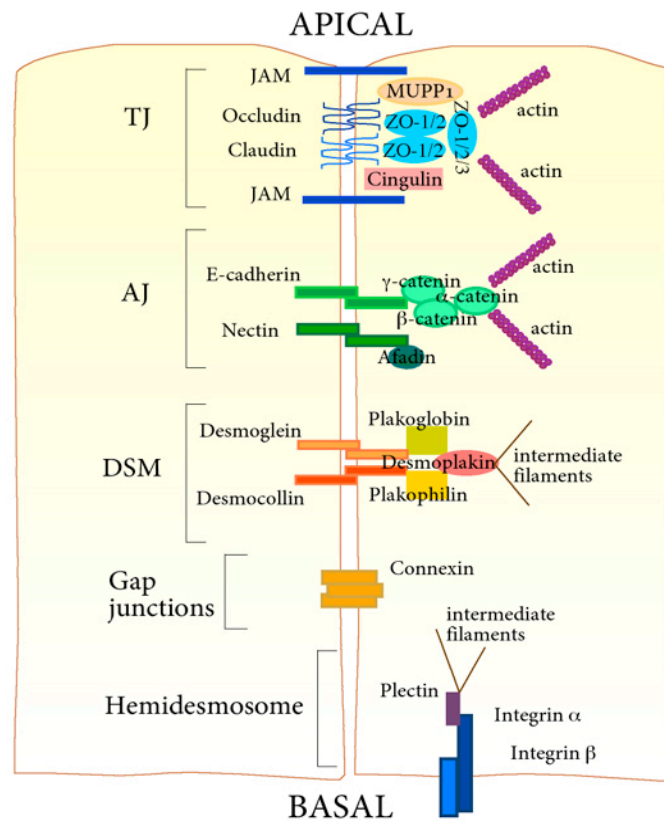


Figure 3. Localization and main constituents of epithelial cell-cell junctions. A schematic illustration of different junctional complexes observed in epithelial cells. Tight junctions (TJs) are the apical most junctions in epithelial cells and consist of transmembrane JAM, occludin and claudin proteins and cytoplasmic proteins ZO-1,-2,-3, cingulin and MUPP. ZO proteins are furthermore shown to bind the actin cytoskeleton. Below TJs are adherens junctions (AJs) consisting of transmembrane E-cadherin and Nectin and intracellular α , β and γ -catenins and afadin. γ -catenin (plakoglobin, also observed in desmosomes) is also binding to actin. Desmosomes (DSM), which are also observed on the lateral side, consist of cadherins, desmoglein and desmocollin, which bind to plakoglobin, plakophilin and desmoplakin on the intracellular side. Desmoplakin furthermore binds desmosomes to intermediate filaments. Below DSMs are found gap junctions consisting of connexin hexamers. On the basal side of epithelial cells are found hemidesmosomes consisting of integrin $\alpha 6 \beta 4$ heterodimer, which on the intracellular side are bound to plectin, which in turn binds to intermediate filaments.

underlying matrix via transmembrane $\alpha 6 \beta 4$ -integrins (Taddei *et al.*, 2003). Inside the cell integrins, which form a heterodimer between α - and β - subunits, bind anchoring protein plectin, and hemidesmosomal plaque proteins BP180 and BP230, which in turn bind to intermediate filaments (Hopkinson and Jones, 2000; Koster *et al.*, 2003; Schaapveld *et al.*, 1998). Moreover, including hemidesmosomal integrin, 24 different $\alpha \beta$ dimers are known. They bind varying ECM substrates, such as collagens, fibronectin and laminins and vary in biological function. For example, integrin- $\beta 1$ appears to be especially important for normal mammary gland function (Hynes, 2002;

Legate *et al.*, 2009). Integrins mediate signals from the ECM to the cells by interacting with several different signaling molecules, including focal adhesion kinase (FAK) and integrin-linked kinase (ILK) and hence activate downstream signaling pathways, such as phosphatidyl inositol-3 kinase (PI3K) and AKT survival pathways (Legate *et al.*, 2009). Finally, integrins are also implicated in cell motility and cancer cell invasion (Huttenlocher and Horwitz, 2011).

2.2 Interactions with basement membrane

Basement membrane (BM) is a specialized form of ECM, which surrounds cells in epithelial tissues as well as endothelial cells in blood vessels. Therefore, BM provides a scaffold to cells and a barrier for transmigration. Physically, BM is a thin, approximately 100 nm dense sheet, composed of a meshwork of insoluble molecules including polymer laminins and crosslinked collagen IV as well as proteoglycans and glycoproteins (Kalluri, 2003; Muschler and Streuli, 2010; Yurchenco, 2011). Epithelial cells secrete BM components, which have the capability of self-assembling to form the BM scaffold (Yurchenco, 2011). Collagen IV is the most abundant protein, although BM is known to be comprised of approximately 50 different proteins. BMs of tissues differ specifically in the expression of laminin trimmers and proteoglycans and nidogen. In the mammary gland, the BM consists mainly of collagen IV, laminins-111, -332, -511 and -521 and proteoglycans, the major proteoglycan being perlecan (Delehedde *et al.*, 2001). Moreover, BM surrounding the mammary ducts has been shown to be of different thickness and composition in comparison to the BM surrounding motile TEBs (Silberstein and Daniel, 1982).

BM components provide not only inert physical support but also maintain specialized epithelial cell functions, such as apico-basal cell polarity. Indeed, polarization of Madin-Darby canine kidney (MDCK) epithelial cells is suggested to be initiated by the organization of polarity by integrin- β 1 binding to extracellular collagens and leading thereafter to polarized deposition of laminin (O'Brien *et al.*, 2001; Yu *et al.*, 2005). Furthermore, after initiation events, the apico-basal polarity and tissue organization is maintained in mammalian and *Drosophila* epithelial cells by directed secretion of BM to the basal side of cells (Daley *et al.*, 2012; Denef *et al.*, 2008) and signaling via Rho and Rac1 pathways (Daley *et al.*, 2012; Myllymaki *et al.*, 2011). Furthermore, it has been demonstrated that binding of epithelial cells to BM induces tissue specific gene expression. In particular, BM has been shown to act in both *in vitro* and *in vivo* experiments as an important regulator of mammary epithelial cell phenotype (Edwards *et al.*, 1998; Edwards *et al.*, 1996; Streuli *et al.*, 1995), even in the absence of cell-cell contacts (Streuli *et al.*, 1991). Additionally, BM contact has been implicated in cell survival (Pullan *et al.*, 1996) and protection from detachment-induced apoptosis (anoikis) of mammary epithelial cells. It has been postulated that attachment to BM induces survival signaling both via integrins and the extracellular signal-regulated kinase (ERK) pathway, which inhibits pro-apoptotic Bcl-2 family members, such as Bim (Mailleux *et al.*, 2007; Reginato *et al.*, 2005; Reginato *et al.*, 2003). Recently,

detachment of ECM and subsequent anoikis has been experimentally shown to result in metabolic stress as well as Hippo pathway signaling (Grassian *et al.*, 2011; Zhao *et al.*, 2012).

The BM can also provide various signaling cues for cells. Proteoglycans, for example, are a group of proteins with diverse functions that can bind and sequester growth factors such as FGF and cytokines, and can thus regulate their availability to cells (Iozzo and Sanderson, 2011; Mongiat *et al.*, 2000). In addition, BM proteins often contain cryptic sites, which are exposed after ECM digestion and can consequently act as pro-migratory, pro-invasive or angiogenic cues (Iozzo *et al.*, 2009; Kalluri, 2003). For example, BM proteoglycan perlecan has been shown to function as an angiogenic factor when intact, yet the C-terminal end of perlecan can act as a pro-angiogenic factor (Iozzo *et al.*, 2009; Zhou *et al.*, 2004). These data suggest that the BM tightly regulates various processes including cell polarity, growth, angiogenesis and cell invasion.

Finally, the BM can be remodeled by various different enzymes both in normal and diseased tissues. MMPs are an evolutionary conserved family of 25 enzymes, which can be divided into soluble MMPs and cell surface attached membrane-type (MT)-MMPs. MMPs are able to degrade various ECM and cell surface molecules and thus aid in tissue remodeling and invasion (Hotary *et al.*, 2006; Liotta *et al.*, 1977; Sternlicht and Werb, 2001) and they have been demonstrated to be especially important in the process of branching morphogenesis (Reviewed in 1.3). However, it is unclear which of these enzymes contribute specifically to degradation of BM collagen IV, although MMP-2 and MMP-9 have been implicated in the process (Hotary *et al.*, 2006; Page-McCaw *et al.*, 2007). Additionally, different serine-proteases have been implicated in remodeling of the BM. For instance, urokinase plasminogen activator (uPA) and tissue plasminogen activator do not directly degrade ECM, but they activate a cascade leading to activation of ECM degrading plasmin and MMPs (Baramova *et al.*, 1997; Mazziere *et al.*, 1997; Ogura *et al.*, 2008). Interestingly, also Hepsin, a serine-protease known to be frequently upregulated in prostate cancer, has been implicated specifically in the degradation of BM component laminin-332 (Dhanasekaran *et al.*, 2001; Tripathi *et al.*, 2008). In contrast to induction of BM disruption, BM can also be remodeled by lysyl oxidase-mediated crosslinking of collagen IV to increase the matrix compliance, which may increase the ability of cancer cells to migrate and invade surrounding tissue (Levental *et al.*, 2009).

2.3 Apico-basal polarity

Cell polarity, the uneven distribution of intracellular organelles, macromolecules and cytoskeleton in the cell is a defining feature of metazoan cells but is observed also in unicellular organisms including yeast and ciliated protozoa (Reviewed by Bryant and Mostov, 2008; Goldstein and Macara, 2007). In multicellular organisms cell polarity has particular importance in establishing correct organization and functionality of

specialized tissues, such as the epithelium. Cells can polarize both along the plane of tissue, defined as planar cell polarity or along the apical-basal axis (Figure 4; Goldstein and Macara, 2007; Wang and Nathans, 2007). Furthermore, cell polarity can be observed in migrating cells, where asymmetry is necessary for directional movements of the cells (Etienne-Manneville, 2008).

Indeed, apico-basal polarity is a defining feature of organized epithelial cells and even though epithelial tissues vary in form and function, the underlying principle of apico-basal polarity is similar. In apico-basally polarized epithelial cells the apical surface, which is oriented towards the lumen, controls exchange and secretion of materials and is in simple epithelia distinguished by structures such as microvilli (Bryant and Mostov, 2008). Lateral surfaces provide contact to adjacent cells via different cell-cell junctions (Reviewed in 2.1) and the basal surface anchors the cells to the ECM and BM. This compartmentalization along the apical-basal axis directs the basic differentiated functions of epithelial cells. For example, molecular asymmetries guide directional secretion in glandular epithelium, such as of digestive enzymes from exocrine pancreas, milk from mammary epithelium, or vectorial transfer of nutrients across the gut epithelium to blood (Bryant and Mostov, 2008). Cell polarity is also linked to regulation of the plane of cell division, as mitotic spindle orientation is coupled to polarity determinants such as E-cadherin and proteins of the PAR polarity complex (den Elzen *et al.*, 2009; Durgan *et al.*, 2011; Hao *et al.*, 2010). Additionally, basal cues also appear to be important for this process, as demonstrated by incorrect plane of cell division of basal mammary epithelial cells in mice lacking integrin- β 1 from myoepithelial cells (Taddei *et al.*, 2008). Orientation of the mitotic spindle is an important feature with regard to cell fate determination and oriented cell division by stem cells.

2.3.1 Core polarity complexes

Formation of polarized epithelial tissues requires the coordinated action of several polarity regulating proteins and protein complexes. The core polarity complexes primarily responsible for the maintenance of cell polarity in epithelial tissues are the apical Crumbs (CRB) complex, the subapical Partitioning defective (PAR) complex and the basolateral Scribble (SCRIB) complex (Figure 4; Reviewed by Macara, 2004; Martin-Belmonte and Mostov, 2008). These polarity complexes are evolutionary conserved and together form a network that establishes and maintains the compartmentalization of a polarized cell.

The PAR family and complex

The PAR family of proteins were originally discovered in a mutation screen for disrupted polarity of the *C. elegans* zygote (Kemphues *et al.*, 1988). Loss of any one of the PAR genes found in the screen led to a failure to asymmetrically organize cell fate determining proteins and RNA to opposing poles of the fertilized zygote. Consequently, the two daughter cells formed after the first mitosis had similar contents and

developmental fate and were hence partitioning defective. The PAR proteins discovered were later found out to be a functionally, but not structurally related group required for the establishment of cell polarity in various cell types (Assemat *et al.*, 2008; Suzuki and Ohno, 2006). Thus far 6 members of the PAR family, PAR-1 to PAR-6, have been discovered and also, mammalian homologues have been identified to all but PAR-2 (Goldstein and Macara, 2007; Macara, 2004). The PAR family consists of two PDZ-domain containing scaffold proteins (PAR-3 and PAR-6; Benton and St Johnston, 2003; Hung and Kemphues, 1999), two serine-threonine kinases (PAR-1 and PAR-4 (Guo and Kemphues, 1995; Lizcano *et al.*, 2004), a RING-finger protein (PAR-2) and a 14-3-3 protein (PAR-5), which binds and sequesters other signaling proteins (Morton *et al.*, 2002). Most of the PAR proteins are asymmetrically distributed in polarized epithelial cells: PAR-3 and PAR-6 are localized apically and PAR-1 and PAR-5 laterally (Goldstein and Macara, 2007). However, PAR-4 and sometimes also PAR-5, are localized uniformly to cell membranes and to some extent also in cytosol, yet they have been shown to contribute to formation of cellular asymmetry in the absence of fixed localization (Baas *et al.*, 2004; Martin and St Johnston, 2003).

The PAR complex (also referred to as Par6 complex) is formed by PAR-3 and PAR-6 together with CDC42 and atypical protein kinase C (aPKC), a serine-threonine kinase also found in the original partitioning defective screen (Joberty *et al.*, 2000; Kemphues *et al.*, 1988). The PAR-6 and PAR-3 proteins do not exhibit enzymatic activity, but as scaffold proteins they regulate their interaction partners by physical binding. For instance, PAR-6 binds to PAR-3 via its PDZ domain, to GTP-bound CDC42 via its central CDC42 and RAC interactive (CRIB) domain, and to aPKC via its N-terminally localized Phox and Bem1p (PB1) domain (Assemat *et al.*, 2008; Joberty *et al.*, 2000; Lin *et al.*, 2000; Noda *et al.*, 2001). This binding of PAR-6 to aPKC is required for aPKC activity (Yamanaka *et al.*, 2001). Furthermore, the interaction between PAR-3 and PAR-6/aPKC is shown to be indispensable for formation and maintenance of cell polarity (Horikoshi *et al.*, 2009). Interestingly, the PAR complex proteins have also been linked to the assembly of TJs. For instance, PAR-3 has been shown to be indispensable for TJ formation, whereas PAR-6 has been shown to negatively regulate the process (Chen and Macara, 2005; Gao *et al.*, 2002; Hirose *et al.*, 2002; Hurd *et al.*, 2003).

The PAR complex and PAR family proteins mainly preserve cell polarity by mutual exclusion. In this process, proteins exclude each other from their respective membrane domains to maintain compartmentalization. For instance, in the PAR complex, aPKC phosphorylates PAR-1 if it is diffused to the apical membrane and thus inhibits the apical spread of basolateral factors (Hurov *et al.*, 2004; Suzuki *et al.*, 2004). In contrast, the basolaterally localized PAR-1 kinase (microtubule affinity regulating kinases MARK1-4 in mammalian cells) is able to phosphorylate PAR-3 if it is diffused away from the apical membrane (Morton *et al.*, 2002). Lateral PAR-5 can then in turn sequester phosphorylated PAR-3 and release it from the membrane to the cytosol, which antagonizes the spread of apical determinants to the basolateral side. Also,

lethal giant larvae (LGL), a member of the basolateral SCRIB complex can antagonize the PAR complex by binding PAR-6/aPKC and thus inhibiting them from interacting with PAR-3 (Yamanaka *et al.*, 2003). Similarly, aPKC can phosphorylate LGL and exclude the SCRIB complex from the apical domain (Betschinger *et al.*, 2003; Tian and Deng, 2008). Taken together, the multiple mechanisms functioning simultaneously in sustaining apico-basal polarity suggest that it is important for the epithelium and thus needs to be appropriately controlled.

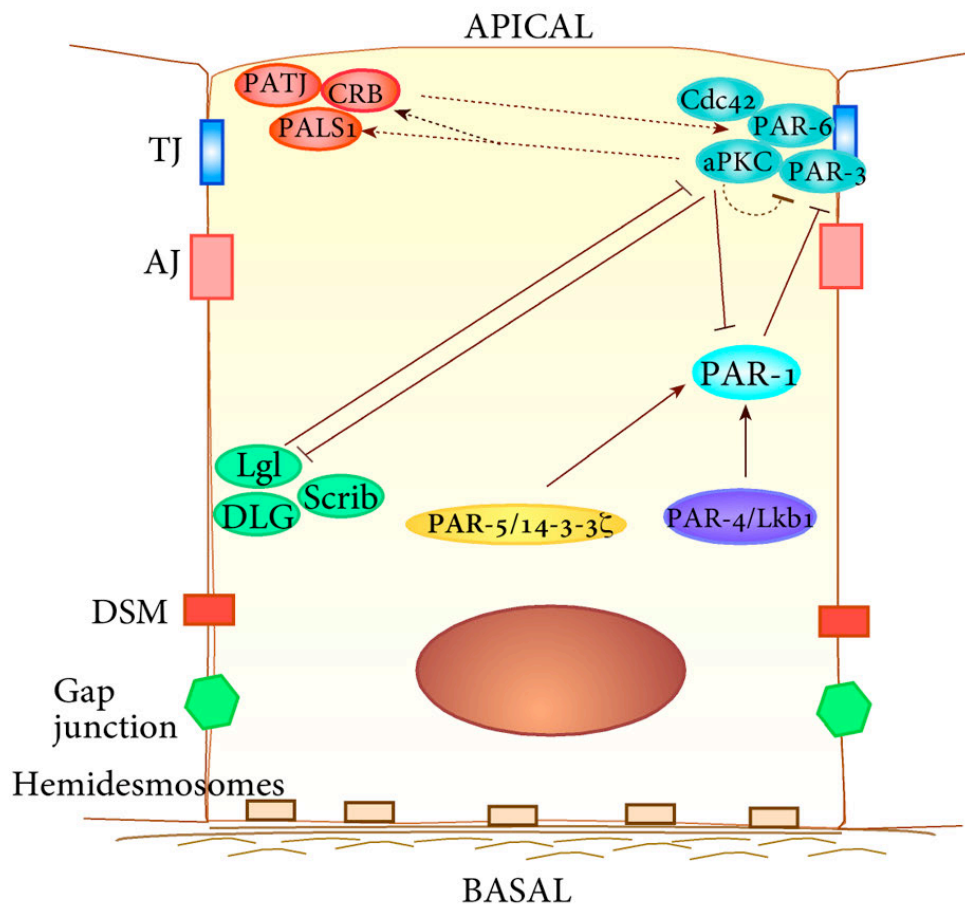


Figure 4. Key polarity proteins and their interactions in epithelial cells. Schematic representation of most the important polarity proteins and protein complexes in the epithelial cell. CRB complex consisting of Crb, PATJ and PALS1, is the apical-most polarity complex localizing at the subapical region. The PAR complex, consisting of PAR-6, PAR-3, aPKC and cdc42 resides in TJs directly below the subapical region. SCRIB complex, consisting of Scrib, LGL and Dlg, is localized at the basolateral membrane. Polarity proteins PAR-1/MARK, PAR-5/14-3-3z and PAR-4/Lkb1 do not have specific localization in the cell, yet they have been shown to have uniform cytoplasmic localization. The figure also summarizes the mutually antagonistic interactions between the different polarity complexes (modified from Goldstein & Macara 2008 and Martin-Belmonte & Perez-Moreno 2012).

CRB complex

The CRB complex consists of Crumbs, Protein associated with lin seven 1 (PALS1), PALS1-associated tight junction protein (PATJ) and multi-PDZ domain protein MUPP (Assemat *et al.*, 2008). CRB complex is localized to the apical-most region above TJs in epithelial cells, and is together with the PAR complex a major apical determinant. Overexpression of transmembrane protein Crumbs leads to expansion of the apical domain and shrinking of the basolateral domain, while loss of Crumbs leads to eradication of the apical domain (Lemmers *et al.*, 2004; Roh *et al.*, 2003; Wodarz *et al.*, 1995). Furthermore, Crumbs, PALS1 and PATJ proteins have all been linked to the formation of epithelial organization and cell polarity in both *Drosophila* and mammalian cells (Knust *et al.*, 1993; Roh *et al.*, 2003; Shin *et al.*, 2005), altogether suggesting an important role for the CRB complex in cell polarity regulation. Also, it seems that the CRB complex is important in the formation of TJs, as overexpression of CRB3 in MCF10A cells normally lacking this protein induces TJ formation (Fogg *et al.*, 2005; Lemmers *et al.*, 2004). Furthermore, PALS1 and PATJ are also required for TJ formation in MDCK cells (Shin *et al.*, 2005; Straight *et al.*, 2004). PALS1 and PATJ/MUPP, which are both cytoplasmic scaffold proteins, have also been shown to directly interact with TJ proteins claudin, occludin and ZO-3 (Assemat *et al.*, 2008).

Interestingly, it has been recently observed that TJ associated polarity proteins PAR-6 and aPKC interact with the CRB complex member PALS1 (Assemat *et al.*, 2008; Hurd *et al.*, 2003; Wang *et al.*, 2004a). Furthermore, there is increasing evidence that the members of the PAR and CRB polarity complexes interact. For example, phosphorylation of Crumbs by aPKC has been shown to be required for Crumbs activity (Sotillos *et al.*, 2004) and Par-3 has been demonstrated to associate with CRB complex (Krahn *et al.*, 2010). Additionally, Crumbs has been shown in *Drosophila* epithelial cells to compete with PAR-6 for binding of Bazooka (Par-3; Morais-de-Sa *et al.*, 2010) and moreover, in the presence of Crumbs aPKC phosphorylates Bazooka leading to dissociation of the Par-3 from the Par-6/aPKC complex. Thus exclusion of Bazooka/Par-3 has been suggested to be required for demarcation of the apical-basal border, at least in *Drosophila* epithelial cells. It is interesting to speculate whether these two apical polarity complexes interact with each other to induce formation of TJs and apical polarity also in mammalian cells.

SCRIB complex

The SCRIB complex, which consists of scribble, discs large (Dlg1-4 in mammalian cells) and Lethal giant larva (Lgl 1-2 in mammalian cells), was originally identified in *Drosophila*. Mutations in all of these genes lead to similar phenotypes characterized by excess proliferation of imaginal discs and visible tumors of larval and adult fly tissues (Bilder *et al.*, 2000; Brumby and Richardson, 2005). Furthermore, loss of *Drosophila* scrib, dlg, or lgl lead to mislocalization of apical domain constituents to the basolateral domain suggesting all of these proteins function in maintenance of cell polarity and

specifically the basolateral identity (Bilder *et al.*, 2000). Indeed, the SCRIB complex proteins are localized to the basolateral region in both *Drosophila* and mammalian cells and are interdependent for correct localization (Assemat *et al.*, 2008; Massimi *et al.*, 2008). SCRIB complex proteins have been postulated to form a complex together, yet evidence from physical binding of the different proteins has been scarce. Scribble has been shown to interact with Dlg in *Drosophila* synapses and with Lgl2 in mammalian epithelial cells (Kallay *et al.*, 2006; Katoh and Katoh, 2004; Mathew *et al.*, 2002) suggesting some protein binding exists between the complex members, however the details of the interactions are yet to be resolved.

As discussed above, SCRIB complex proteins maintain the basal identity of cells through Lgl binding to PAR-6/aPKC, which inhibits the function of the PAR complex and excludes Par-3 from the complex (Yamanaka *et al.*, 2003). Furthermore, AJs have been demonstrated to be essential for the correct localization of Scribble and Dlg1, which have been shown to colocalize with E-cadherin in human cervical epithelium and mouse intestine (Navarro *et al.*, 2005; Yoshihara *et al.*, 2011). These data imply that AJ formation at the lateral membrane is needed for the maintenance of the basal compartment.

2.3.2 Other cell polarity regulators

In addition to the above reviewed cell polarity proteins and polarity complexes, other factors have also been implicated in the formation and maintenance of cell polarity. Rho GTPases for example, are an important protein family linked to the regulation of apico-basal cell polarity as well as polarized cell functions such as cytoskeletal dynamics, vesicle trafficking and cytokinesis (reviewed by Tervonen *et al.*, 2011). Specifically, RhoA, Rac1 and CDC42, which have been demonstrated to have a role in regulating the formation of stress fibers, lamellopodia and filopodia, have also been implicated in the initiation and maturation of AJs (Kuroda *et al.*, 1997). Rac1 is recruited by E-cadherin to newly formed cell-cell junctions and CDC42 is shown to be activated during formation of E-cadherin-mediated cell contacts (Kim *et al.*, 2000b; Kuroda *et al.*, 1997). Also, RhoA appears to be required for E-cadherin based cell adhesion and to cause accumulation of TJ components ZO-1 and occludin at cell-cell junctions (Braga *et al.*, 1997; Gopalakrishnan *et al.*, 1998), yet conversely, RhoA activity is also shown to be inhibited at AJs (Noren *et al.*, 2001). Therefore, activation of Rac1 and CDC42 and inhibition of RhoA are necessary steps towards formation of cell polarity following cell-cell contact formation. Also, overexpression of dominant-negative Rac1 or hyperactivation of the RhoA-Rho kinase (ROCK) pathway are able to invert the polarity of MDCK 3D cysts leading to formation of the apical compartment to the basal side and vice versa (Liu *et al.*, 2007; Yu *et al.*, 2008), hence demonstrating a more direct role in cell polarity for these GTPases.

Proteins are sorted to specific locations in the cell by vesicular trafficking pathways using the cytoskeleton as a guide. Sorting of proteins to different locations is achieved

by different protein motifs and attachment of post-translational modifications. For example, the formation of distinct apical and basal plasma membranes requires the differential sorting of BM constituents and receptors to the basal side (Orlando and Guo, 2009). The sorting can thus maintain epithelial organization by sustaining differential apical and basal compartments. Indeed, in *Drosophila*, faulty localization of BM proteins to apical membrane in *crag* and *scarf* mutant epithelium leads to loss of epithelial integrity (Denef *et al.*, 2008; Sorrosal *et al.*, 2010). Also, in *Drosophila* epithelium mutations in Rab and Syntaxin, the core components of endocytic vesicle trafficking, results in disruption of apico-basal polarity (Lu and Bilder, 2005). Furthermore, in MDCK cells Rab GTPase-dependent apical trafficking of PAR complex proteins is essential for the formation of apical surface lumen in 3D structures (Bryant *et al.*, 2010). Interestingly, basal polarity determinant LGL has also been implicated in polarized exocytosis (Gangar *et al.*, 2005; Zhang *et al.*, 2005) suggesting that LGL might also contribute to cell polarity via regulation of vesicular trafficking.

Interestingly, in addition to differential localization of proteins, polarized epithelial cells appear to exhibit lipid asymmetries. In MDCK cysts it has been demonstrated that phosphatidyl-inositol 4,5 biphosphate (PIP₂) is accumulated on the apical lipid bilayer and phosphatidyl-inositol (3,4,5) triphosphate (PIP₃) is accumulated on the basal domain (Gassama-Diagne *et al.*, 2006). The lipid asymmetry is generated by the interplay between PI3K and by its negative regulator phosphatase and tensin homolog (PTEN): PI3K activity leads to formation of PIP₃ whereas PTEN dephosphorylates PIP₃ and PIP₂ (Leslie *et al.*, 2008). Importantly, the lipid asymmetry seems to have a functional role in the maintenance of cell polarity, as insertion of PIP₂ to the basal domain of MDCK cysts led to redistribution of apical determinants to the basal compartment and thus inversion in the cyst polarity – similar defects were observed if PIP₃ was inserted into the apical membrane (Gassama-Diagne *et al.*, 2006; Martin-Belmonte *et al.*, 2007). Furthermore, loss of PTEN inhibited formation of PIP₂ asymmetry and apical surface and cyst lumen (Martin-Belmonte *et al.*, 2007) via mechanisms involving Cdc42 activation and subsequent recruitment of PAR-6/aPKC complex to the apical pole (Martin-Belmonte *et al.*, 2007; Takahama *et al.*, 2008). Taken together, in addition to designated polarity proteins, other factors including PI3K, PTEN and Rho GTPases also appear to be important in the formation and maintenance of cell polarity.

3. TUMOR SUPPRESSION BY EPITHELIAL INTEGRITY GENES

Cancer progresses towards malignancy through gradual acquisition of genetic lesions. These lesions allow uncontrolled cell proliferation by providing independency over external growth stimuli, by sustaining proliferative signaling and by evading normal growth suppression as well as apoptosis (Hanahan and Weinberg, 2011). These changes will eventually lead to the expansion of cancer cells. In addition to

unrestrained proliferation, cancer cells often acquire other traits, which expedite tumor growth, such as limitless replication potential, capability to sustain angiogenesis and to invade surrounding tissues. Furthermore, formation of solid tumors requires, in addition to cancer cells, involvement of and interaction with many other types of cells including stromal fibroblast, endothelial cells and pericytes. Additionally, different types of immune cells, such as lymphocytes, macrophages and mast cells are implicated in tumorigenesis (Hanahan and Weinberg, 2011; Pietras and Ostman, 2010). Therefore, development of tumors is a complex process and transition from benign lesions to invasive and metastatic disease can be a slow process.

Various tumor suppressor mechanisms have developed to control unscheduled cell proliferation induced by activating mutations in proto-oncogenes and inactivating mutations in tumor suppressor genes, thereby inhibiting tumor formation (Hanahan and Weinberg, 2011; Lee and Muller, 2010). Classical tumor suppressor genes are characterized by their ability to induce programmed cell death (apoptosis), inhibit cell cycle progression, cell growth or to activate DNA repair mechanisms (Lee and Muller, 2010; Macleod, 2000). Indeed, prototypical tumor suppressor genes inhibit the function of proto-oncogenes, which transduce growth signals from cell surface growth factor receptors to nucleus in order to induce growth related gene expression and cell cycle progression. As postulated by Knudson (Knudson, 1971), the progression towards malignancy usually requires inactivation of tumor suppressor genes located at both chromosomes. Frequently, one inactivated allele is inherited while the other one is inactivated somatically during tumor progression. Deletion of the remaining allele is called loss of heterozygosity (LOH). Some tumor suppressors however, exhibit haploinsufficiency, where one copy of the gene is not sufficient to fulfill the normal function of the gene, and haploinsufficient tumor suppressors are therefore compromised by a single hit (Payne and Kemp, 2005).

Tumor suppressor genes can be divided into two main categories: the gatekeepers, such as p53 and pRb, which directly inhibit cell growth and induce apoptosis of tumor cells, and the caretakers, such as DNA repair enzymes, which prevent accumulation of mutations (Kinzler and Vogelstein, 1997; Oliveira *et al.*, 2005). Also, a third category of tumor suppressor genes, termed landscapers, has been suggested (Kinzler and Vogelstein, 1998). The landscaper genes, such as Lkb1, function in a more indirect manner compared to gatekeeper and caretaker genes. Landscapers have been proposed to act on the ECM, secreted factors and adhesions of the cells rather than on cell growth. It has been suggested that landscapers might contribute to the malignant transformation of adjacent epithelial cells via modulation of the microenvironment (Kinzler and Vogelstein, 1998). Hence, it is interesting to speculate whether genes regulating epithelial structure would also fall into this category of tumor suppressors.

3.1 Tumor suppression in breast cancer

Breast cancer is the most common cancer among women and the second most common cause of cancer deaths (www.who.int). Hence, approximately every 9th woman will develop breast cancer during her lifetime. The risk of developing breast cancer is increased with age and in individuals with a family history of breast cancer. It is difficult to single out other major factors contributing to breast cancer risk, yet life time exposure to estrogen seems to play a role. Generally, early menarche, late menopause and oral contraceptives as well as hormone replacement therapy are considered to be predisposing factors. In contrast, full term pregnancy at an early age and breast-feeding has been shown to protect from breast cancer (Akbari *et al.*, 2011; Butt *et al.*, 2009; Reeves *et al.*, 2011). More recently, it was established that women with higher mammographic density, which reflects the amount of ECM and cellularity in the breast, have a higher lifetime risk of developing breast cancer (Boyd *et al.*, 2011). In addition, numerous lifestyle factors including cigarette smoking, obesity and alcohol consumption, contribute to the overall risk of developing breast cancer (Khan *et al.*, 2010).

The majority of breast cancers occur due to mutations in somatic cells. They include both mutations activating proto-oncogenes as well as mutations inactivating tumor suppressor genes (Hanahan and Weinberg, 2011; Lee and Muller, 2010). Several oncogenes relevant to the progression of sporadic breast cancer, such as ErbB2 (Her-2), c-Myc and Cyclin D1, have been found, but in contrast, tumor suppressors specific for sporadic breast cancer have not been identified. However, tumor suppressors implicated in several types of cancers have also been identified in breast cancers. For instance, the p53 tumor suppressor gene has been shown to be mutated in approximately 20-30% of sporadic breast cancers (Gasco *et al.*, 2002; Hollstein *et al.*, 1991).

Additionally, it has been estimated that 10-25% of breast cancer cases are attributable to inherited factors (Reviewed by Nathanson *et al.*, 2001; Ripperger *et al.*, 2009). Hereditary breast cancers on the other hand, have been suggested to be due to a small number of highly penetrant germline mutations and a larger number of low-risk mutations in tumor suppressor genes (Nathanson *et al.*, 2001; Ripperger *et al.*, 2009). Breast cancer 1 and 2 (*BRCA1* and 2) were the first breast cancer susceptibility genes discovered, and today they are known to account for approximately 20% of familial breast cancer cases (Futreal *et al.*, 1994; Miki *et al.*, 1994). Carriers of these mutations have a lifetime risk of 60-80% of developing breast cancer. Also, several low-risk germline mutations in tumor suppressor genes predisposing to breast cancer are known to date. They include known tumor suppressor genes such as *TP53*, *LKB1* and *PTEN*, which are respectively associated with Li-Fraumeni, Peutz-Jeghers and Cowden cancer predisposition syndromes, however, they only account for a minority of familial breast cancer cases (Hearle *et al.*, 2006; Malkin *et al.*, 1990; Saal *et al.*, 2008). Recently, genome wide association studies (GWAS) have been used to try to

understand genetic factors underlying predisposition to breast cancer. Indeed, new loci encoding putative breast cancer susceptibility including genes such as *FGFR2* and *MAP3K1* have been identified (Easton *et al.*, 2007). Intriguingly, *FGFR2* encodes a receptor tyrosine kinase implicated in mammary branching morphogenesis and *MAP3K1* is a serine-threonine kinase implicated in activation of pro-survival pathways, such as ERK. However, these genes are mutated in approximately 10-15% of breast cancers and hence the majority of the genes predisposing to breast cancer are still to be identified.

3.2 Epithelial integrity in tumor suppression in *Drosophila*

In *Drosophila*, tumor suppressor genes have been classified into two main categories according to the effects these inactivating mutations cause to epithelial integrity (Hariharan and Bilder, 2006). Using larval imaginal discs as a model epithelium, it has been shown that inactivation of a hyperplastic tumor suppressor gene (hTSG), such as *pten*, *tsc1* or *tsc2* or one of the components of the growth regulatory *hippo/salvador/lats* pathway, leads to benign growth of the imaginal disc epithelial cells (Harvey *et al.*, 2003; Huang *et al.*, 1999; Tapon *et al.*, 2001; Wu *et al.*, 2003b). Nevertheless, these cells remain as an epithelial monolayer and eventually differentiate into adult tissue. In contrast, loss of neoplastic tumor suppressor genes (nTSGs), such as *scribble* (*scrib*), *discs large 1* (*dlg*) or *lethal (2) giant larvae* (*lgl*), induces malignant growth, prevents the imaginal cells from organizing into an epithelial monolayer and induces differentiation defects (Bilder and Perrimon, 2000; Gateff, 1994; Oppen *et al.*, 1987; Woods and Bryant, 1991). Therefore, in *Drosophila* intact epithelial structure can act as a barrier against tumor formation. Interestingly, whereas the numerous hTSGs identified are regulators of cell growth, cell cycle and apoptosis, most of the few known nTSGs regulate cell polarity (Reviewed by Hariharan and Bilder, 2006). More specifically, *lgl*, *dlg* and *scrib* are members of the basal SCRIB complex. More recently, new nTSGs, such as *avl*, *rab5*, *tsq101*, *vps25* and *vps45* that have been discovered appear to control endocytosis (Lu and Bilder, 2005; Menut *et al.*, 2007; Moberg *et al.*, 2005; Vaccari and Bilder, 2005), which is critical for the localization of cell polarity determinants. Furthermore, recent data has shown that some hTSGs are also connected to cell polarity. Apical polarity protein Crumbs has been shown to regulate Hippo signaling in *Drosophila* (Chen *et al.*, 2010), via Hippo-dependent control of TGF- β 1 signaling (Varelas *et al.*, 2010).

In addition to the ability of nTSGs to disrupt the epithelial integrity of *Drosophila* imaginal disc epithelium, it was demonstrated in two independent studies that inactivating mutations in any of the nTSG genes induced metastatic tumor growth in combination with activated Ras (Brumby and Richardson, 2005; Pagliarini and Xu, 2003). In contrast, inactivation of hTSGs or expression of Ras alone induced only non-metastasizing tumors. Also, Par-3 homologue *bazooka*, PALS1 homologue *stardust* and *cdc42* were similarly found to cooperate with Ras (Pagliarini and Xu, 2003). Thus, it appears that cell polarity and epithelial structure determinants can keep oncogenic

signaling in check and furthermore, that epithelial structure can act as a powerful tumor suppressor mechanism.

3.3 Epithelial integrity linked genes in human cancers

Loss of cell polarity and cell-cell contacts are common in advanced solid cancers and often indicate aggressive disease and high metastatic potential of the primary tumor. For instance, loss of epithelial adhesion molecules E-cadherin and ZO-1 are common lesions in human cancers (Tervonen et al., 2011). However, whether genes regulating epithelial integrity have a causal role in the initiation of human cancers is less clear.

At present, many human orthologues of the *Drosophila* hTSGs are implicated in cancer progression, including TSC1/2, PTEN and NF2/Merlin (Reviewed in Tervonen et al., 2011). However, it is not known whether orthologues of nTSGs or other epithelial integrity regulators are equally important in human cancers. Instead, there is emerging evidence linking other cell polarity and epithelial structure regulators to human cancers. For example, PAR-6 β , one of the three PAR-6 isoforms has been shown to be amplified in breast cancers (Nolan *et al.*, 2008) and is found in breast cancer cells. Moreover, overexpression of PAR-6 β has been shown to confer growth factor-independent cell proliferation (Nolan *et al.*, 2008) and the PAR-6 pathway has been implicated in BRCA1-associated breast cancer (Viloria-Petit *et al.*, 2009). Also, both isoforms of aPKC, aPKC ι /lambda and aPKC ζ , have been shown to be either amplified or overexpressed in several forms of cancers including lung, ovarian, colon, liver and pancreatic cancer (Eder *et al.*, 2005; Evans *et al.*, 2003; Murray *et al.*, 2004; Regala *et al.*, 2005; Tsai *et al.*, 2000). Interestingly, it seems that mislocalization of aPKC from the cell-cell borders into the cytoplasm is an important aspect of aPKC deregulation in cancers (Regala *et al.*, 2005). In contrast to PAR-6/aPKC, PAR-3 has been shown to be frequently lost in cancers (Fang and Xu, 2001; Rothenberg *et al.*, 2010; Zen *et al.*, 2009).

Likewise, proteins residing in other polarity complexes are implicated in human cancer. For instance, CRB complex proteins PATJ and MUPP1 are targeted by viral oncoproteins leading to their degradation. Both are also targeted by adenoviral E4-ORF and human papillomavirus (HPV) E6 (Lee *et al.*, 2000; Storrs and Silverstein, 2007). Moreover, loss of Crb3 has also been shown to lead to contact-inhibited growth and tumorigenesis (Karp *et al.*, 2008). The CRB complex might regulate cell growth by physically binding mTOR inhibiting complex TSC2 via PATJ (Massey-Harroche *et al.*, 2007), or as recently demonstrated in *Drosophila*, by regulation of the Hippo tumor suppressor pathway (Chen *et al.*, 2010). Additionally, SCRIB complex proteins Scribble, DLG and LGL are all targeted by HPV E6 and E7 oncoproteins for degradation (Gardioli *et al.*, 1999; Nakagawa and Huibregtse, 2000). Mutations in the SCRIB complex proteins are rare, yet Scribble mutations have been found from renal cell carcinoma and carcinomas of the breast and colon (Dalgliesh *et al.*, 2010; Sjoblom *et al.*, 2006) while Dlg1 mutations are found in mammary ductal carcinoma (Fuja *et al.*, 2004).

However, mislocalization of the SCRIB complex proteins has been shown to occur often in cancer cells. For example, Scribble was recently shown to be mislocalized and downregulated in human breast cancers (Zhan *et al.*, 2008) and Dlg and Scribble were shown to be mislocalized in colon carcinoma cells (Gardiol *et al.*, 2006). Furthermore, the mislocalization of SCRIB proteins has been implicated in their diminished activity (Zhan *et al.*, 2008). Similarly, reduced LGL expression has been reported in several human tumor types (Grifoni *et al.*, 2007). Taken together, these data indicate that loss of human homologs of the *Drosophila* nTSGs are evident in human cancers.

3.4 Tumor suppressor LKB1

The LKB1/STK11 (Liver kinase B1, serine-threonine kinase 11) gene within chromosomal locus 19p13 encodes for a tumor suppressor kinase demonstrated to have a role in regulation of epithelial cell polarity. Germline mutations in tumor suppressor LKB1 are associated with autosomal dominant inherited Peutz-Jeghers polyposis syndrome (PJS) (OMIM: 175200), with inactivating mutations identified in practically all PJS patients (Aretz *et al.*, 2005; Hemminki *et al.*, 1998). PJS is characterized by mucosal pigmentation and benign hamartomatous polyps occurring throughout the gastrointestinal tract (Katajisto *et al.*, 2007). Moreover, patients with PJS have a significantly higher risk of developing cancer than the general population (Giardiello *et al.*, 1987; Hearle *et al.*, 2006). PJS patients frequently develop cancers of the gastrointestinal tract, pancreas, breast and ovaries (Hearle *et al.*, 2006; Mehenni *et al.*, 2006) and in particular, the female PJS patients have an approximately 30% risk of developing breast cancer before the age of 60 (Hearle *et al.*, 2006).

Recently, mutations in the LKB1 gene have also been observed in a subset of sporadic tumors. Most frequently, LKB1 mutations have been found in non-small cell lung carcinoma (NSCLC) and in cervical cancer (Ji *et al.*, 2007; Sanchez-Cespedes *et al.*, 2002; Wingo *et al.*, 2009), where approximately 30% and 20% of cases respectively harbor mutations. Biallelic inactivation of LKB1 is observed in cervical cancers, but not in NSCLC cases (Gao *et al.*, 2010a; Ji *et al.*, 2007; Wingo *et al.*, 2009). LKB1 mutations have also been identified in melanomas and cancers of the pancreas, liver, breast and endometrium, although in much smaller numbers (Contreras *et al.*, 2008; Forster *et al.*, 2000; Guldberg *et al.*, 1999; Kim *et al.*, 2004; Su *et al.*, 1999), supporting a role for LKB1 as a tumor suppressor in sporadic cancers as well. Additionally, reduced expression levels of Lkb1 have been reported in a range of cancers, including endometrial, pancreatic and breast cancer (Contreras *et al.*, 2008; Fenton *et al.*, 2006; Morton *et al.*, 2010; Sahin *et al.*, 2003), moreover, in breast cancer, reduced Lkb1 levels have been shown to correlate with shortened survival (Fenton *et al.*, 2006; Shen *et al.*, 2002). However, in breast cancer Lkb1 mutations appear to be rare, yet reduced Lkb1 expression can be attributable to epigenetic silencing, such as promoter methylation, which has been reported in papillary breast tumors (Esteller *et al.*, 2000; Fenton *et al.*, 2006). Furthermore, indirect mechanisms leading to inactivation of LKB1 signaling have also been proposed. In melanoma cells for instance, mutated B-

RAF leads to phosphorylation of LKB1 in a manner which inhibits it from activating downstream targets (Zheng *et al.*, 2009).

Functional data from mouse models further emphasize the role of Lkb1 as a tumor suppressor (summarized in Ollila and Makela, 2011; and Shackelford and Shaw, 2009). Mice with tissue specific heterozygosity or loss of *Lkb1* develop tumors in the pancreas, mammary gland, liver and endometrium (Contreras *et al.*, 2010; Contreras *et al.*, 2008; Hezel *et al.*, 2008; McCarthy *et al.*, 2009; Nakau *et al.*, 2002). While homozygous deletion of *Lkb1* in the endometrium results in rapidly growing invasive carcinomas (Contreras *et al.*, 2010; Contreras *et al.*, 2008), homozygous deletion of *Lkb1* in the mammary gland results in formation of mammary tumors in only 19% of animals with long latency (McCarthy *et al.*, 2009). Furthermore, recent studies also show that loss of Lkb1 can increase the tumorigenic potential of oncogenic lesions such as K-Ras or loss of tumor suppressor PTEN as well as predispose to chemical carcinogenesis (Gurumurthy *et al.*, 2008; Huang *et al.*, 2008; Ji *et al.*, 2007; Morton *et al.*, 2010). Altogether, these results indicate that whether loss of Lkb1 promotes tumor formation on its own or predisposes to it strongly depends on the tissue context.

3.4.1 Lkb1 signaling

Lkb1 protein is a 51 kDa serine-threonine kinase expressed ubiquitously in early embryogenesis and homozygous inactivation of Lkb1 results in embryonic lethality due to severe neural tube defects and vascular abnormalities (Ylikorkala *et al.*, 2001). Later in embryonic development, Lkb1 expression becomes restricted in the heart, kidney, pancreas and gastro-intestinal tract (Luukko *et al.*, 1999) and in adult tissues, Lkb1 expression is highest in the majority of epithelial cells, in reproductive organs and in skeletal muscle cells and glia cells (Conde *et al.*, 2007; Rowan *et al.*, 2000).

The kinase activity of Lkb1 in mammalian cells requires heterotrimeric complex formation with pseudokinase Ste20-related adaptor protein (STRAD) and scaffold protein mouse protein 25 (MO25) (Baas *et al.*, 2003; Boudeau *et al.*, 2003). Binding to STRAD and MO25 renders Lkb1 active and furthermore, regulates Lkb1 protein stability and localization (Boudeau *et al.*, 2003; Dorfman and Macara, 2008). In overexpression studies, Lkb1 has been shown to localize to the nucleus, yet localization of endogenous Lkb1 is primarily cytoplasmic (Boudeau *et al.*, 2003; Tiainen *et al.*, 2002). Moreover, cytoplasmic localization has been demonstrated to be essential for the tumor suppressor functions of Lkb1 such as cell cycle regulation (Nezu *et al.*, 1999; Tiainen *et al.*, 2002). Interestingly, it was recently reported that Lkb1 together with STRAD co-localizes with E-cadherin at AJs in polarized MDCK cells and also that this co-localization is needed for Lkb1 kinase activity (Sebbagh *et al.*, 2009).

Lkb1 has been implicated in the control of cellular metabolism and cell polarity by functioning as the upstream kinase of AMP-activated protein kinase (AMPK) and 13

related kinases via direct phosphorylation in several different cell types (Figure 5; Hawley *et al.*, 2003; Lizcano *et al.*, 2004; Shaw *et al.*, 2004; Woods *et al.*, 2003). The AMPK serine-threonine kinase family belongs to the kinase subfamily of Calcium/calmodulin-dependent protein kinases (www.kinase.com) and consists of AMPK, MARKs 1-4, AMPK-related kinase 5 (ARK5, NUA1), SNF/AMPK related kinase (SNARK, NUA2), Brain specific kinases (BRSK) 1-2, salt-induced kinases (SIK) 1, QIK (SIK2), QSK (SIK3) and Snf-related serine/threonine kinase (SNRK) (Lizcano *et al.*, 2004).

One of the most widely studied Lkb1 targets is AMPK, which is essential for maintenance of cellular energy homeostasis in normal and transformed cells (Reviewed by Alexander and Walker, 2011). AMPK is heterotrimer consisting of a catalytic α subunit and regulatory β and γ subunits. AMPK is activated by a drop in ATP and increase in AMP levels via binding of AMP to the AMPK γ subunit but also requires direct phosphorylation of AMPK α at T172 by either Lkb1 or Calmodulin-dependent protein kinase kinase (CaMKK) both *in vitro* and *in vivo* (Hawley *et al.*, 2003; Shaw *et al.*, 2004; Woods *et al.*, 2003). Hence, metabolic stress leading to increased consumption or decreased production of ATP leads to activation of AMPK. To accommodate a drop in ATP levels, AMPK inhibits protein synthesis by inhibition of mammalian target of rapamycin (mTOR) signaling by phosphorylating tuberous sclerosis (TSC2/TSC1) complex and mTOR binding partner, regulatory associated protein of mTOR (raptor) (Gwinn *et al.*, 2008; Inoki *et al.*, 2003). These proteins are important regulators and transducers of growth factor and nutrient signaling, which are deregulated in most human cancers.

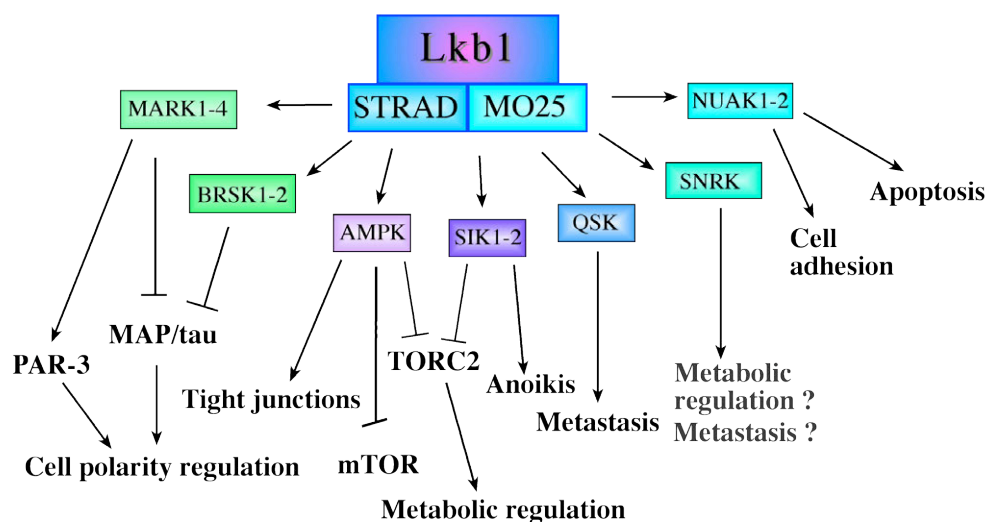


Figure 5. Downstream effectors of tumor suppressor Lkb1. The figure shows downstream targets of serine-threonine kinase Lkb1 and their respective downstream effectors implicated in various cellular processes. MARK1-4 and BRSK1-2 kinases are implicated in regulation of cell polarity, whereas AMPK1-2 are implicated both in cell polarity and regulation of cell metabolism. Other Lkb1 downstream targets are also shown to regulate several processes. SIK1-2 have been implicated in regulation of metabolism and anoikis, NUA1-2 in cell adhesion and apoptosis, and SNRK possibly to metabolic regulation and metastases. Also, QSK has been linked to metastases (modified from Katajisto *et al.* 2007 and Hezel & Bardeesy 2008).

Lkb1 has been shown to induce p53 activity and p21 mRNA expression (Karuman *et al.*, 2001; Tiainen *et al.*, 2002) and furthermore, AMPK has been shown to modulate p53-induced apoptosis by direct phosphorylation and activation of p53 (Imamura *et al.*, 2001; Jones *et al.*, 2005). Additionally, the Lkb1-AMPK pathway has been linked to autophagy, a process where cells break down their own components using lysosomal pathways when in unfavorable energy conditions. For instance, the Lkb1-AMPK pathway has been implicated in the phosphorylation and stabilization of p27 under energy stress conditions, leading to an increase in cell survival through autophagy (Liang *et al.*, 2007). Also, both Lkb1 and AMPK have been shown to induce and lead to phosphorylation of forkhead box 3a (FOXO3a) transcription factor, which has been shown to play an important role in metabolism and autophagy (Greer *et al.*, 2007; Zhong *et al.*, 2008), thus further underlining the role of AMPK in regulation of both cellular energy levels and cell survival. In addition to AMPK, the less well characterized kinases NUA1 and 2, QSK, SNRK and SIKs also appear to have functions in stress responses and metabolism, in addition to their role in metastasis (Alessi *et al.*, 2006; Cheng *et al.*, 2009).

3.4.2 Regulation of cell polarity by Lkb1

The first evidence demonstrating a role for Lkb1 in the regulation of cell polarity came from a screen for mutants disrupting the first asymmetric cell divisions of *C. elegans* embryo (Kemphues *et al.*, 1988). PAR-4, the *C. elegans* orthologue of Lkb1, was among other PAR genes shown to be required for anterior-posterior polarity (Figure 6). More recently, it was reported that loss of Lkb1 from the *Drosophila* oocyte leads to disruption of anterior-posterior cell polarity. Similarly, in follicular epithelial cells, loss of Lkb1 has been shown to disrupt apico-basal polarity as manifested by disorganization of the epithelium and mislocalization of polarity markers including aPKC (Martin and St Johnston, 2003). Likewise, loss of Lkb1 in *Drosophila* retinal cells leads to disruption of polarity and disarrangement of membrane domains (Amin *et al.*, 2009). In mammalian cells, Lkb1 has been shown to be linked to polarity regulation of single intestinal epithelial cells, where forced expression of Lkb1 leads to formation of cell polarity and positioning of the TJ marker ZO-1 in the absence of cell-cell contacts (Baas *et al.*, 2004). Also, Lkb1 has been implicated in polarity of mouse oocytes (Szczepanska and Maleszewski, 2005) and furthermore, loss of Lkb1 was demonstrated to be required for polarity *in vivo* in pancreatic acinar cells (Hezel *et al.*, 2008) and of cortical neurons (Barnes *et al.*, 2007; Shelly *et al.*, 2007).

Several Lkb1 downstream kinases have been implicated in Lkb1-induced cell polarity in a highly context specific manner (Figure 6). For instance, energy sensor AMPK has been shown to be critical for formation of cell polarity and TJs (Zhang *et al.*, 2006; Zheng and Cantley, 2007), as disruption of AMPK function by dominant-negative AMPK delays TJ formation after depletion of calcium and subsequent re-addition in

MDCK cells. Intriguingly, Lkb1-AMPK signaling was demonstrated to be essential for maintenance of cell polarity during energy deprived conditions. In *Drosophila* follicular epithelial cells, both Lkb1 and AMPK null mutants exhibited normal epithelial architecture in nutrient rich environment but when they were deprived from sugars in culture media, apico-basal cell polarity was disrupted (Mirouse *et al.*, 2007). Moreover, myosin regulatory light chain has been suggested to mediate these effects (Lee *et al.*, 2007). These data suggest that the Lkb1-AMPK axis is especially important in sustaining cell polarity when energy is reduced. Interestingly, Lkb1 downstream target NUA1 was also shown to be associated with myosin phosphatases and to control cell adhesion (Zagorska *et al.*, 2010). Hence, Lkb1 seems to control cell adhesion via two independent mechanisms.

PAR-1/MARK has been implicated as a downstream effector of Lkb1 in apico-basal polarity for example in pancreatic acinar cells *in vivo* (Granot *et al.*, 2009). PAR-1 was also identified in the same *C. elegans* partitioning defective screen as Lkb1/PAR-4 (Kemphues *et al.*, 1988), and has thereafter been strongly linked to regulation of cell polarity. For instance, PAR-1 was shown in *Drosophila* to phosphorylate Par-3 to retain it in the apical pole of the oocyte (Morton *et al.*, 2002) and to regulate the anterior-posterior gradient of microtubules (Tian and Deng, 2009). In mammalian cells, the PAR-1 homologs MARKs also contribute to cell polarity by phosphorylating microtubule-associated proteins (MAPs) such as tau (Tassan and Le Goff, 2004) that have been shown to affect polarized protein trafficking. Also, Par-1b/MARK-2 has been demonstrated to control RhoA activity and actin cytoskeleton re-organization (Yamashita *et al.*, 2011). Intriguingly, Par-1 was shown to be targeted by *Helicobacter*

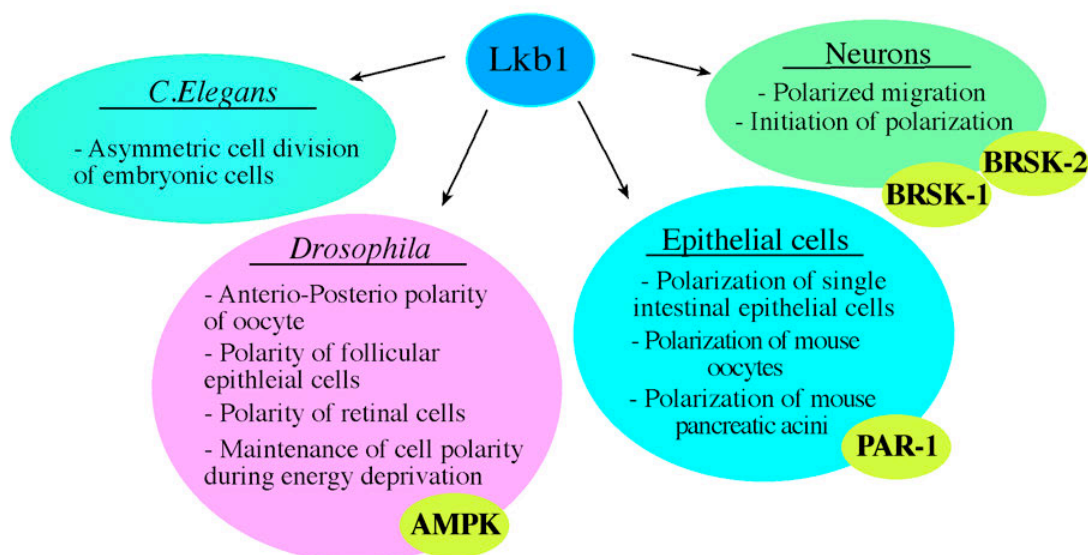


Figure 6. Lkb1 in the regulation of cell polarity. The figure summarizes the evidence linking Lkb1 to cell polarity regulation in *C. elegans*, *Drosophila* as well as mammalian epithelial and neuronal cells. Highlighted are known downstream targets, through which Lkb1 has been shown to regulate cell polarity in different systems.

pylori virulence factor CagA (Saadat *et al.*, 2007) consequently leading to disruption of cell polarity. Finally, Lkb1 has been shown to induce polarized migration of neurons and promote axon initiation during neuronal polarization through downstream kinases BRSK1 and -2 (also known as SAD-A and -B), which have also been demonstrated to phosphorylate MAPs and hence contribute to cell polarity (Asada *et al.*, 2007; Barnes *et al.*, 2007; Shelly *et al.*, 2007).

4. THE C-MYC ONCOGENE

c-Myc proto-oncogene was first discovered as the cellular homologue of avian myelocytomatosis retrovirus oncogene *v-myc* (Sheiness and Bishop, 1979). c-Myc is a member of the MYC gene family comprised of MYC, MYCN and MYCL1, which encode structurally related proteins. These proteins are thought to exert their main functions via transcription factor activity and they have been linked to a variety of cellular functions including cell growth, proliferation, self-renewal, apoptosis, metabolism and blocking differentiation (Reviewed by Larsson and Henriksson, 2010; Meyer and Penn, 2008). Furthermore, a strong link between MYC family genes and human cancers has been established (Larsson and Henriksson, 2010).

4.1 Structure and regulation of c-Myc

The MYC family proteins are essential for cell growth and proliferation and thus their expression is tightly regulated in normal physiological context (Figure 7). The expression of MYCN and MYCL1 genes is normally limited to embryonic development while c-Myc encoding MYC gene is expressed ubiquitously during development and in the proliferating cells of an individual. Furthermore, c-Myc has been shown to be indispensable for embryonic development as mice lacking both c-Myc alleles die at embryonic day E9.5-E10.5 (Davis and Halazonetis, 1993).

The MYC gene encodes for a 64.5 kDa transcription factor containing a carboxyl terminal basic-helix-loop-helix-leucine-zipper (bHLHZip) domain and a transactivation domain (TAD) in its amino terminus. Also, c-Myc contains four conserved elements termed the Myc Box (MB) I-IV, two of which reside within the TAD. Through the bHLHZip domain, c-Myc binds and forms a heterodimeric complex with its bHLHZip interaction partner Max and is thus able to bind CACGTG E-box DNA sequences (Blackwood and Eisenman, 1991). Furthermore, the interaction between c-Myc and Max is essential for c-Myc-induced biological functions as well as cellular transformation (Amati *et al.*, 1993a; Amati *et al.*, 1993b). c-Myc-induced transcription can be antagonized by Mxd (also known as Mad) family proteins, which bind and heterodimerize with Max and bind the same DNA sequences as Myc/Max heterodimers (Ayer *et al.*, 1993). Additionally, the Mnt family of bHLHZip proteins is also known to repress Myc target genes by similarly binding to Max. Thus, Mdx and

Mnt proteins form together a network modulating c-Myc functions (Larsson and Henriksson, 2010).

The Myc/Max complex has been shown to activate transcription by a number of mechanisms, which include recruiting additional factors to DNA thereby contributing to promoter activation. Chromatin remodeling has been established as one such mechanism, as c-Myc has been demonstrated to bind transactivation/transformation-associated protein (TTRAP) via its MB II region, thus recruiting histone acetylase complexes to chromatin for activation of transcription (McMahon *et al.*, 1998; McMahon *et al.*, 2000). Similarly, c-Myc has been shown to interact with other proteins, such as INI/hSnf5, TIP48 and TIP49, which have also been implicated in chromatin remodeling (Cheng *et al.*, 1999; Wood *et al.*, 2000). Furthermore, N-Myc was previously shown to induce widespread alterations in chromatin structure, partly via histone acetyltransferase GCN5 (Knoepfler *et al.*, 2006). Additionally, other mechanisms of transcriptional activation by c-Myc have also been described: c-Myc can, for instance, promote RNA polymerase II C-terminal phosphorylation, mRNA cap methylation (Cowling and Cole, 2007), transcriptional pause release (Rahl *et al.*, 2010), and recruitment of elongation factor p-TEFb (Eberhardy and Farnham, 2002).

While the Myc/Max heterodimer has largely been linked to transcriptional activation, c-Myc is also known to induce transcriptional repression of certain target genes (Eilers and Eisenman, 2008; Kleine-Kohlbrecher *et al.*, 2006). Both E-box dependent and independent mechanisms for transcriptional repression have been described (Li *et al.*, 1994; Wanzel *et al.*, 2003). For instance, the Myc/Max complex has been demonstrated to interact with factors, such as Miz-1, NFY and SP1, which are directly bound to DNA and consequently contribute to transcriptional repression (Kleine-Kohlbrecher *et al.*, 2006). Additionally, activation of microRNAs, which can simultaneously target multiple mRNAs by affecting their stability and repressing translation is yet another layer of complexity to c-Myc-induced transcriptional repression (Bui and Mendell, 2010). In addition, it has become increasingly evident that c-Myc induces and represses its target genes both in a cell type dependent as well as independent manner (Eilers and Eisenman, 2008). c-Myc has been estimated to bind approximately 10-15% of mammalian genomic loci, and a significant amount of these loci are also estimated to be modulated by c-Myc at the transcriptional level (Fernandez *et al.*, 2003; Li *et al.*, 1994; Zeller *et al.*, 2006). Altogether, thus far as many as ~1700 putative c-Myc target genes have been found using various techniques (Dang *et al.*, 2006; Zeller *et al.*, 2003) and have been catalogued (www.myccancergene.org). However, it is still unclear and remains for future studies to explore, which of these genes are genuine transcriptional targets of c-Myc.

Expression of the c-Myc gene is controlled by proliferative and anti-proliferative signals, as mitogenic signaling quickly induces MYC mRNA in quiescent cells (Kelly *et al.*, 1983) and growth inhibitory signaling downregulates c-Myc (Dean *et al.*, 1986). In differentiated tissues, c-Myc signaling is modulated by numerous internal and

external factors such as EGF, platelet derived growth factor (PDGF), interferons and interleukins, estrogen, and the NF- κ B pathway. These signals, in turn, function via eg. ER, Stat's, and transcription factors E2F, Oct, ETS, AP1 and TCF among others (Reviewed by Levens, 2010). Furthermore, c-Myc expression is regulated post-translationally by protein and mRNA stability. Both c-Myc mRNA and protein were shown to have short half-lives in quiescent cells yet their expression was demonstrated to be stable in cycling cells (Dani *et al.*, 1984; Hann and Eisenman, 1984; Thompson *et al.*, 1985). c-Myc has two major stability regulating phosphorylation sites within the MB I domain, serine-62 (S62) and threonine-58 (T58) (Henriksson *et al.*, 1993; Pulverer *et al.*, 1994). S62 is phosphorylated by ERK, and leads to increased stability of c-Myc protein (Pulverer *et al.*, 1994; Sears *et al.*, 2000). In contrast, T58 is phosphorylated by glycogen synthase kinase 3 β (GSK3 β) and targets c-Myc for degradation (Sears *et al.*, 2000). Upstream signaling via Ras oncoprotein has been shown to maintain c-Myc stabilization, since it is able to modulate the phosphorylation status of both S62 and T58 by activation of ERK and inhibition of GSK3 β (Gregory *et al.*, 2003; Sears *et al.*, 2000). Additionally, other mechanisms leading to c-Myc stability in malignant cells have been described. These include PP2A inhibition by Cancerous inhibitor of PP2A (CIP2A) and increased CIP2A expression in head and neck squamous cell carcinoma and colon carcinoma (Junttila *et al.*, 2007), as well as loss of Axin-1 in certain human breast cancers and breast cancer cell lines (Arnold *et al.*, 2009; Zhang *et al.*, 2011). Also, the I κ B kinase and NF- κ B essential modulator have been suggested to induce S62 phosphorylation and degradation of c-Myc (Kim *et al.*, 2010; Yeh *et al.*, 2011).

4.2 Cell cycle and growth related functions of c-Myc

c-Myc has a fundamental role in cell cycle progression and cell growth. It is essential for progression from G0/G1 to S phase and as described above, is induced promptly in response to growth promoting signals and downregulated by anti-growth signals. The promotion of cell cycle progression by c-Myc is postulated to be due to transcriptional activation of several target genes such as E2F1 and E2F2, Cyclins D1, D2, E1 and A2, cyclin dependent kinase 4 (CDK4) and cell division cycle 25A (CDC25A) and repression of cell cycle inhibitory genes GADD45 and GADD153 as well as CDK inhibitors p21, p27 and p15INK4A via interaction with Miz-1 or FOXO3a (Figure 7; Dang *et al.*, 2006; Staller *et al.*, 2001; Yang *et al.*, 2001). Furthermore, c-Myc has been shown to increase protein synthesis (Mateyak *et al.*, 1997) and ribosomal biogenesis related proteins and rRNA (Gomez-Roman *et al.*, 2003; Kim *et al.*, 2000a), presumably to accommodate increased need for resources in cell growth. Consequently, c-Myc expression levels have also been implicated in body size control via regulation of cell size and number (Kim *et al.*, 2000a; Trumpp *et al.*, 2001). Also, deletion of c-Myc from neuronal progenitor cells, hematopoietic stem cells or from embryonic stem cells leads to depletion of differentiated cell pools and further underlines that c-Myc is indispensable for growth and expansion of developing tissues (Baudino *et al.*, 2002;

Wilson *et al.*, 2004). Cell cycle and growth control engage also several metabolic pathways, many of which c-Myc has been shown to regulate. These include glycolysis, glutaminolysis, and lipid metabolism (Dang *et al.*, 2006; Zeller *et al.*, 2006) further underlining that activation of c-Myc induces a comprehensive change in cell growth regulation.

Several studies emphasize the fact that whereas c-Myc upregulation is needed for cell cycle progression, c-Myc downregulation is required for cells to exit the cell cycle and differentiate (Bar-Ner *et al.*, 1992; Delgado *et al.*, 1995; Freytag *et al.*, 1990). Furthermore, the majority of Mxd family proteins antagonizing c-Myc are upregulated during differentiation (Queva *et al.*, 1998). Conversely, c-Myc has also been reported not to be downregulated during induced differentiation of some cell types including erythroid differentiation (Delgado *et al.*, 1995; Lerga *et al.*, 1999). In many systems c-Myc expression is able to inhibit premature differentiation or entirely block differentiation (Bar-Ner *et al.*, 1992; Dmitrovsky *et al.*, 1986; Prochownik and Kukowska, 1986), but in hematopoietic stem cells, human and mouse epidermis and in the mammary gland, c-Myc has been demonstrated to stimulate differentiation (Arnold and Watt, 2001; Blakely *et al.*, 2005; Wilson *et al.*, 2004). The differences between how various systems respond to c-Myc activation most likely reflect the different tissue types and developmental stages. However, several of the studies where c-Myc has been shown to inhibit differentiation have been conducted by overexpression of ectopic c-Myc, suggesting that the expression levels of c-Myc affect the cellular response. In addition, it is plausible that differentiation is a fail-safe mechanism protecting tissues from c-Myc hyperactivity. Indeed, evidence supporting both hypotheses exist, as it has been reported that activation of c-Myc at low levels in various tissues leads to increased proliferation, and high-level activation to increased proliferation accompanied with apoptosis (Murphy *et al.*, 2008). Similarly, activation of c-Myc in mouse epidermis at different levels (Berta *et al.*, 2010) leads to increased proliferation regardless of the intensity of c-Myc activation, yet differentiation occurred only with higher levels of activity. Lastly, c-Myc has recently been shown to play a key role in self-renewal of human and mouse stem and progenitor cells and furthermore, c-Myc has been implicated to be one of the genes needed for reprogramming of differentiated murine and human cells into pluripotency (Takahashi *et al.*, 2007; Takahashi and Yamanaka, 2006).

4.3 Apoptotic functions of c-Myc

In addition to induction of cell growth and proliferation, c-Myc can also induce apoptosis in a wide variety of cell types (Askew *et al.*, 1991; Evan *et al.*, 1992). The apoptosis-inducing function of c-Myc has been described as a fail-safe mechanism operating in response to increased proliferation (Lowe *et al.*, 2004). Hence, hyperproliferation driven by deregulated c-Myc can be restrained by increased apoptosis and therefore, c-Myc-induced apoptosis has been regarded as a major inhibitor of c-Myc-induced tumorigenesis (Hemann *et al.*, 2005; Pelengaris *et al.*,

2002b). Importantly, c-Myc has also been shown to sensitize cells to a range of apoptosis inducing agents such as genotoxic agents, serum or growth factor deprivation and death receptors ligands (Reviewed in Nieminen *et al.*, 2007). Whether cells then respond to c-Myc induction by entering an apoptosis sensitized state or directly undergoing apoptosis has been suggested to be attributable to the level of c-Myc activation as well as other survival/death signals available for the cells (Murphy *et al.*, 2008).

Tumor suppressor p53 is regarded as one of the major mediators of cell cycle arrest or apoptosis in response to DNA damage, oncogenic or other types of stress. c-Myc has been demonstrated to induce apoptosis either in a p53 dependent or independent manner according to the cell type (Hsu *et al.*, 1995; Sakamuro *et al.*, 1995) and furthermore, there has been shown to be cross talk between p53 and c-Myc apoptotic pathways (Hoffman and Liebermann, 2008). p53 has been demonstrated to induce apoptosis by induction of pro-apoptotic transcriptional target genes, such as Puma, Bax and Noxa (Miyashita *et al.*, 1994; Miyashita and Reed, 1995; Oda *et al.*, 2000). p53 is normally kept at low levels in cells by degradation triggered by Mouse double minute-2 (Mdm2), which in turn is negatively regulated by p19ARF. It has been shown that c-Myc activation leads to elevated p19ARF expression, which then activates p53 (Hermeking and Eick, 1994; Zindy *et al.*, 1998). Interestingly, oncogenic c-Myc and ARF can both cooperate in the induction of apoptosis in response to p53 activation by ATM kinase (Pauklin *et al.*, 2005; Pusapati *et al.*, 2006), which regulates cellular response to double-strand breaks in DNA and can also regulate p53. Hence, it has been suggested that in cancer cells c-Myc activity causes accumulation of DNA damage, which leads to activation of the ATM pathway (Felsher and Bishop, 1999; Pusapati *et al.*, 2006). Additionally, it has been shown that p53 and c-Myc have opposing effects on cyclin-dependent kinase inhibitor p21, an important regulator of cell cycle arrest. While p53 can activate p21, c-Myc represses it and thus, activation of c-Myc can drive the cell towards apoptosis instead of growth arrest in response to p53 activation (Seoane *et al.*, 2002; Wu *et al.*, 2003a). Interestingly, it was demonstrated in the *Em-Myc* lymphoma model that overexpression of T58A mutant c-Myc leads to activation of p53, yet induction of apoptosis was impaired due to a failure to activate pro-apoptotic Bcl-2 family member and BH3-only protein Bim (Hemann *et al.*, 2005). These results thus imply that c-Myc induced apoptosis requires not only p53, but also the cooperation of other apoptosis pathways.

In addition to the p53 pathway, c-Myc has been described to sensitize cells to apoptosis mediated through death receptors. Upon c-Myc activation, primary and non-transformed cells have been shown to become sensitized to TNF- α , CD95L and TRAIL-induced apoptosis (Hueber *et al.*, 1997; Klefstrom *et al.*, 1994; Ricci *et al.*, 2004). All of these apoptosis inducing ligands, or death ligands, bind to their cognate receptor at the cell surface and transduce apoptotic signals inside the cell via activation of a death inducing signaling complex (DISC). DISC activation then leads to

either activation of survival pathways, downstream executioner caspases or the mitochondrial apoptosis pathway. c-Myc has been demonstrated to enhance the apoptosis pathways downstream of death receptors at several different points. For example, c-Myc activation leads to reduced levels of caspase-8 inhibitor Fas-associated death domain like IL-1 β converting enzyme (FLIP), and increases the expression levels of TRAIL receptor DR5 and CD95L (Brunner *et al.*, 2000; Ricci *et al.*, 2004; Wang *et al.*, 2004b). Additionally, c-Myc has been shown to inhibit TNF-activated prosurvival c-Jun kinase and NF- κ B (Klefsstrom *et al.*, 1997) as well as inhibit TRAIL induction of anti-apoptotic Mcl-1 and cIAP2 by inhibition of NF- κ B (Ricci *et al.*, 2007).

Activation of the mitochondrial apoptosis pathway downstream of death receptors has recently been demonstrated to be important in apoptosis regulation by c-Myc. Mitochondria have an essential role in apoptosis as, for instance, the release of cytochrome c from the mitochondria leads to activation of downstream caspases responsible for the execution of apoptosis. Release of cytochrome c to the cytosol is a consequence of disruption of mitochondrial outer membrane permeability (MOMP), a process which can be induced by pro-apoptotic Bcl-2 family proteins Bax and Bak (Wei *et al.*, 2001), and which is tightly regulated by other Bcl-2 family members (Martinou and Youle, 2011). Indeed, c-Myc-induced sensitization to apoptosis was shown to be mediated by cytochrome c release from the mitochondria (Iaccarino *et al.*, 2003; Juin *et al.*, 1999) and there is also an increasing amount of evidence that c-Myc induces apoptosis and apoptotic sensitization by affecting the Bcl-2 family members. For instance, c-Myc-induced apoptosis has been shown to be mediated by Bax (Juin *et al.*, 2002). c-Myc appears to upregulate Bax transcriptionally, but also via mechanisms involving oligomerization of Bax proteins at the mitochondrial outer membrane (Annis *et al.*, 2005; Mitchell *et al.*, 2000). Similarly, loss of Bax blocks the ability of c-Myc to induce apoptosis in the *E μ -Myc* mouse lymphoma model (Eischen *et al.*, 2001b), in the mouse mammary tumor virus (MMTV)-Myc mammary tumor model (Jamerson *et al.*, 2004) and in a pancreatic β -cell model (Dansen *et al.*, 2006) suggesting that Bax is needed for c-Myc induced apoptosis also *in vivo*. Furthermore, in the lymphoma model a subset of the lymphomas lacking Bax led to an increase in the levels of anti-apoptotic Bcl-2 and Bcl-X_L independently of p53 mutations (Eischen *et al.*, 2001b). Correspondingly, tumors in Bim deficient *E μ -Myc* mice were lacking p53 or p19ARF mutations (Egle *et al.*, 2004) suggesting altogether that p53 mutations are redundant in the context of apoptosis block on the level of Bcl-2 regulation. Otherwise, loss of other pro-apoptotic Bcl-2 family members, such as Bim, Noxa, or Puma, have also been shown in the *E μ -Myc* lymphoma model to inhibit c-Myc-induced apoptosis and accelerate lymphomagenesis (Egle *et al.*, 2004; Hemann *et al.*, 2005). Additionally, c-Myc-induced apoptosis is shown to be modulated by the expression of anti-apoptotic Bcl-2 or Bcl-X_L. c-Myc has been demonstrated to dampen the expression levels of Bcl-2 or Bcl-X_L, most likely via direct and indirect mechanisms (Eischen *et al.*, 2001a; Eischen *et al.*, 2001b). Also, overexpression of Bcl-2 or Bcl-X_L increase the rate of c-

Myc-induced tumorigenesis in the mammary gland and in pancreatic β -cells (Jager *et al.*, 1997; Pelengaris *et al.*, 2002b), and comparably, endogenous Bcl-2 and Bcl-X_L have been shown to be required for c-Myc-induced tumorigenesis (Kelly *et al.*, 2011). To summarize, c-Myc moderates the level of both anti- and pro-apoptotic Bcl-2 family members, which appear to be key mediators of c-Myc-induced tumorigenesis.

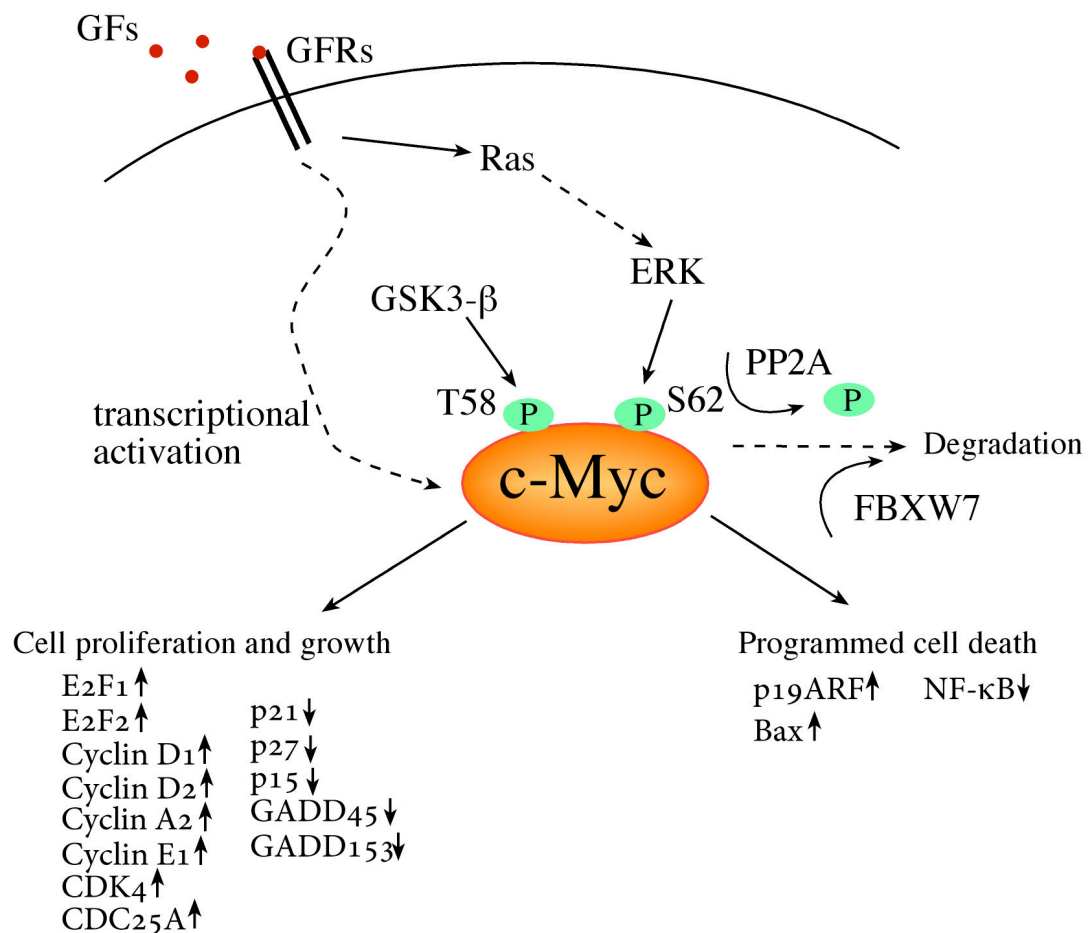


Figure 7. Regulation and downstream targets of c-Myc. A schematic representation of pathways regulating c-Myc and c-Myc downstream targets. Myc function is regulated upstream by growth factor signaling, for example via activation of Ras, which can lead to both transcriptional activation of c-Myc as well as to stabilization of the Myc protein. Transcriptional targets of c-Myc implicated in induction of cell proliferation and growth include E2F1-2 and CyclinD1-2 as well as p21 and p27, which are downregulated by Myc. Transcriptional targets implicated in apoptosis include p19 and Bax, which are upregulated by Myc, and NF- κ B which is downregulated.

4.4 c-Myc in breast cancer

The first evidence for the involvement of c-Myc in human cancer was observed from Burkitt's lymphoma, where the chromosomal locus 8q24 containing the *MYC* gene was translocated into the vicinity of immunoglobulin heavy or light chain on chromosomes 2, 14 or 22 (Reviewed by Pelengaris *et al.*, 2002a). Furthermore, this translocation was shown to contribute to the deregulation of c-Myc and to progression of the disease both in humans and in mouse models. Deregulation of c-Myc has thereafter been shown in several types of human cancers including cancers of the breast, lung, colon, and ovaries (Albihn *et al.*, 2010; Pelengaris *et al.*, 2002a). Similarly, deregulation of *MYCN* and *MYCL* have also been observed in cancers. *MYCN* overexpression is frequently observed in neuroblastomas, small cell lung carcinoma and in tumors of neuroendocrine origin, whereas *MYCL* deregulation is observed almost exclusively in small cell lung carcinomas (Dang, 2012; Westermarck *et al.*, 2011) thus further underlining the role of the *MYC* family genes in cancer progression.

Amplification of the *MYC* locus occurs in approximately 20% of breast cancer cases (Aulmann *et al.*, 2006; Corzo *et al.*, 2006), yet the overexpression level of *MYC* mRNA and protein are seen in 20-45% of cases, depending on the study (Bieche *et al.*, 1999; Chrzan *et al.*, 2001; Naidu *et al.*, 2002). Furthermore, *MYC* amplification specifically correlates with aggressive disease and poor clinical outcome in patients (Aulmann *et al.*, 2006). Also, *MYC* amplification is detected more often in high-grade ductal carcinoma *in situ* (DCIS) and in invasive carcinoma (Blancato *et al.*, 2004; Rummukainen *et al.*, 2001) in contrast to benign or hyperplastic lesions. Recently, it was demonstrated that c-Myc is highly expressed in breast tumors classified in the basal-like subgroup and that also, a *MYC* gene expression signature was observed in basal-like breast cancers (Sorlie *et al.*, 2001). Furthermore, it has been shown that *MYC* is frequently amplified in hereditary breast cancers with *BRCA1* mutation or sporadic cancers with *BRCA1* inactivation (Grushko *et al.*, 2004; Wei *et al.*, 2005). Interestingly, both mutations often associate with basal-like, ER α negative breast cancer as well as aggressive disease.

To study the molecular mechanisms underlying c-Myc-induced mammary tumorigenesis, mouse models overexpressing c-Myc under mammary specific promoters have been created. Two independent groups have created mice expressing c-Myc under the whey acidic protein (WAP) promoter (Sandgren *et al.*, 1995; Schoenenberger *et al.*, 1988), which is activated in differentiated alveolar cells during late pregnancy and lactation (Campbell *et al.*, 1984). Also, mouse models where c-Myc is activated under the glucocorticoid-induced MMTV promoter or under tetracycline (tet) activatable MMTV-tet promoter have been developed (D'Cruz *et al.*, 2001; Stewart *et al.*, 1984). Thus, all of the models require pregnancy-induced activation of the promoters and multiple pregnancies for tumor formation. The first reports suggest that the latency of c-Myc-induced mammary tumorigenesis varies from 6-9 months, yet the penetrance of the tumors also varied markedly from 50-100%

(Sandgren *et al.*, 1995; Schoenenberger *et al.*, 1988). A more recent report using conditionally active c-Myc demonstrated that the tumor latency for mammary tumors was around 3,5 months after the first pregnancy (D'Cruz *et al.*, 2001). The tumors induced by c-Myc have all been shown to be adenocarcinomas and according to a recent report, Wap-Myc tumors can be classified to a subgroup of luminal tumors, although a subset of these tumors also exhibited basal characteristics (Herschkowitz *et al.*, 2007). In accordance, MMTV-Myc tumors have been shown to exhibit genetic heterogeneity (Andrechek *et al.*, 2009), most likely attributable to other genetic lesions arising during the tumorigenic process.

Interestingly, c-Myc has been shown to cooperate in mammary tumorigenesis with a variety of different factors. For example, c-Myc-induced tumorigenesis is augmented by overexpression of H-Ras, TGF α , ErbB2, and Bcl-2 (Amundadottir *et al.*, 1996; Jager *et al.*, 1997; Sinn *et al.*, 1987). In all cases the incidence of the tumors was increased and tumor formation accelerated. Interestingly, c-Myc has been suggested to cooperate with endogenous Ras in tumor formation (Andrechek *et al.*, 2009; D'Cruz *et al.*, 2001). In the tet-inducible Myc model, switching off the conditionally active c-Myc led to regression of half of the c-Myc-induced tumors, while half of the tumors continued to grow (D'Cruz *et al.*, 2001). These tumors had acquired an active mutation in the K-Ras2 gene, and thus were resistant to regression and continued growing. These data imply that mutations rendering the Ras pathway active are preferred in c-Myc-induced tumorigenesis. The specific molecular mechanisms behind the cooperation are still unclear, yet activation of the Ras pathway could confer resistance to c-Myc-induced apoptosis or lead to stabilization of c-Myc by phosphorylation of the S62 residue (Sears *et al.*, 2000). Finally, c-Myc was recently shown to cooperate in mammary tumorigenesis with loss of cell polarity regulator Scribble, which was shown to inhibit c-Myc-induced apoptosis (Zhan *et al.*, 2008). Additionally, c-Myc induced mammary tumors were shown to lack Scribble, implying loss of Scribble is selected for during c-Myc-induced tumorigenesis. Altogether, these data suggest that c-Myc alone is not a strong inducer of mammary tumors, however it is instead able to strongly promote tumorigenesis in the context of other lesions.

II AIMS OF THE STUDY

1. To investigate how epithelial integrity modulates the dual proliferation and apoptotic functions of c-Myc and to examine the molecular mechanisms underlying c-Myc-induced sensitization to apoptosis in 2D and 3D.
2. To explore the role of Lkb1 in the regulation of epithelial integrity in mammary epithelial cells and whether c-Myc and Lkb1 cooperate in mammary tumorigenesis *in vivo*.
3. To characterize the role of human homologs of 65 *Drosophila* cell polarity and epithelial integrity regulators in the regulation of 3D mammary epithelial acinar morphogenesis and in oncogenic cooperation with c-Myc.

III MATERIALS AND METHODS

The materials and methods used in this study are listed here and are described in detail in the original publications, which are here referred by Roman numerals.

MATERIALS

Mouse lines	Description	Source or reference	Used in
FVB	Inbred mouse strain	Harlan / Meilahti animal facility	III
Wap-Myc (FVB.Cg-Tg(Wap-Myc)212Bri/J)	Mouse line expressing c-Myc under the Whey acidic protein (wap) promoter	Jax Laboratories (stock #002677)	III
Wap-Cre (B6.Cg-Tg(Wap-Cre)11738Mam/JKwJ)	Mouse line expressing Cre enzyme under the Whey acidic protein (wap) promoter	Jax Laboratories (stock #008735)	III
Lkb1 flox	Mouse with conditional Lkb1 alleles deletable by Cre enzyme	Dr. Ron de Pinho (Univ of Texas MD Anderson Cancer Center)	III

Cells	Description	Source or reference	Used in
MCF10A	Non-transformed human mammary epithelial cell line	American type culture collection (ATCC)	I,II, IV
HMEC-hT	Primary human mammary epithelial cells immortalized by hTERT	ATCC, immortalized by author	I
MRC5-hT	Primary human lung fibroblast cells immortalized by hTERT	ATCC, immortalized by author	II
MMEC	Primary mouse mammary epithelial cells	Prepared by author	III
Phoenix Amphi	Retroviral packaging cell line	Dr. Garry Nolan	I, II
293fT	Human embryonal kidney cell line transformed with SV40 Large T antigen, used for lentivirus production	ATCC	I-IV

Viral vectors	Description	Source or reference	Used in
pBABE MycER	Retroviral expression vector containing c-Myc fused with hormone binding domain of estrogen receptor (ER)	Dr. Gerard Evan (University of Cambridge)	I,II, IV
pBABE MycΔER	Retroviral expression vector containing c-Myc with deletion in aminoacids 106-143 fused with hormone binding domain of ER	Dr. Gerard Evan (University of Cambridge)	I,II
pWZL hTERT	Retroviral expression vector containing catalytic subunit of human telomerase enzyme	Dr. Martin McMahon (USCF) and Dr. Robert Weinberg (Whitehead Institute/MIT)	I,II
pBABE Hygro Bcl-X _L	Retroviral expression vector containing human Bcl-X _L and hygromycin selection marker	Dr. Gerard Evan (University of Cambridge)	II

pC4M-Fv2E-caspase 8	Protease domain of caspase-8 cloned into a FKBP variant construct allowing ectopic dimerization	Prepared in Klefström laboratory	II
pC4M-Fv2E-mut caspase 8	Protease domain of catalytic site C->A mutant caspase-8	Prepared in Klefström laboratory	II
pLenti6/V5 DEST	Lentiviral expression vector (empty control) with V5 tag	Invitrogen	III
pLenti6 HPNv5	Lentiviral expression vector carrying human Hepsin with C-terminal V5 tag (Human ORFeome Collaboration, clone ID 100009774)	Prepared in Klefström laboratory	III
pENTR-H1	Modified entry vector for Gateway cloning	Biocentrum Helsinki Genome Biology Unit	I,II, IV
pDSL_hpUGIH	Lentiviral shRNA vector containing GFP marker and ires hygromycin selection marker	Alliance for Cellular Signalling (AfCS)	I,II, IV
pLentiLox3.7 (pLL3.7)	Lentiviral shRNA vector containing GFP marker	Dr. Luk Van Parijs	I,II
pLKO.1-puro	Lentiviral shRNA vector containing puromycin selection marker	The RNAi Concoction (TRC), Sigma	III, IV
pGIPZ	Lentiviral miR-30 based shRNA vector containing GFP marker	Open Biosystems	IV

shRNA vectors	Antisense sequence (5'-3')	Source or reference	Used in
pLL3.7 shControl	TTAGTAGAGATCTATGAGA	Prepared in Klefström laboratory	I,II, III
pLL3.7 shCaspase-8	CAGGGTCATGCTCTATCAGATTT	Prepared in Klefström laboratory	I,II
pLL3.7 shLkb1-1	CCCAAGGCTGTTGTGTGAAT	Prepared in Klefström laboratory	III
pLL3.7 shLkb1-2	GCAGAAGATGTATATGGTGAT	Prepared in Klefström laboratory	III
pLKO.1 non-target shRNA	CAACAAGATGAAGAGCACCAA	TRC library, # SHC002	III
pLKO.1 shHepsin	CGACTTCTACGGAATCAGAT	TRC library, #TRC0000304737	III
pDSL_UGIH shCtrl	CTGGTCGTTCTCGAAGAA	Prepared in Klefström laboratory	I,II, IV
pDSL_UGIH shLkb1-1	TCTTCTTGAGGATGTTGAC	Prepared by author	I,IV
pDSL_UGIH shLkb1-2	ATGACTGTGGTGCCGTAAT	Prepared by author	I
pDSL_UGIH shBim	TGTTGATTTGTCACAATC	Prepared in Klefström laboratory	I
pDSL_UGIH shBid	ACAGAATCCGCACTGTGCT	Prepared in Klefström laboratory	I, II
pDSL_UGIH shBak	CATAGCGTCGGTTGATGTCGT	Prepared in Klefström laboratory	II
pDSL_UGIH shGSK3B	GAAACATTGGGTTCTCCTC	Prepared by author	IV
pDSL_UGIH shYAP1-1	GATGAACGCTTCTGTCTCC	Prepared by author	IV
pDSL_UGIH shFUZ1-1	GATTCTGCCAAACATGTG	Prepared by author	IV
pDSL_UGIH shFAT4-3	TCTGTCTTTGGAAATGAGC	Prepared by author	IV
pDSL_UGIH shDVL3-1	GATTCAAGCGATTCTGTG	Prepared by author	IV
pDSL_UGIH shDVL3-2	TGTAGTGGTGGCTAGAAGC	Prepared by author	IV
pDSL_UGIH shDVL3-3	ATGTATTTGGGAATCACCA	Prepared by author	IV
pDSL_UGIH shFZD1-1	CTCAAGCCAGGAAAGAAAG	Prepared by author	IV
pDSL_UGIH shMOESIN-1	CTTATTGAGCTGAGTTTG	Prepared by author	IV
pDSL_UGIH shMOBK1A-1	TTATTCTCCATTACTTGG	Prepared by author	IV
pDSL_UGIH shMOBK1A-2	CTTATTCTCCATTACTTG	Prepared by author	IV
pDSL_UGIH shGSPM2-1	TGTGTGTTTCCAAGATTC	Prepared by author	IV

pDSL_UGIH shNF2-2	TATAAACCACCATATGCTC	Prepared by author	IV
pDSL_UGIH shDAG1-2	GAAGTAGCTGCTCTGTGTG	Prepared by author	IV
pDSL_UGIH shCNNTB1-3	GATGAGCTTGCTTTCTTGG	Prepared by author	IV
pDSL_UGIH shMAPK10-2	CTCCATCCTCGTCTGTCA	Prepared by author	IV
pDSL_UGIH PAR6a-2	ATCATGTCCGTCACCTTGGT	Prepared by author	IV
pDSL_UGIH shPAR6b-2	TATAGGAAGGACAAACACC	Prepared by author	IV
pDSL_UGIH shPar6g-2	GACATTGCTGGACATGTGC	Prepared by author	IV
pDSL_UGIH shPAR6g-3	AGACATTGCTGGACATGTG	Prepared by author	IV
pDSL_UGIH shSTAU2-2	TCAGTCAAAGCTTTATTGG	Prepared by author	IV
pDSL_UGIH shIKK α -1	GTCTCTCCACCAGTTTCCT	Prepared by author	IV
pDSL_UGIH shIKK α -2	AGTCTCTCCACCAGTTTCC	Prepared by author	IV
pDSL_UGIH shEZRIN-1	AGTCAGGTGCCTTCTGTGTC	Prepared by author	IV
pDSL_UGIH shTMOD3-3	TACATTGGTTGGATTGGT	Prepared by author	IV
pDSL_UGIH shMLLT4-3	CTGTGTTCTGCTTTCTTGC	Prepared by author	IV
pDSL_UGIH shMAPK11-3	GTACACTTCGCTGAAGTCC	Prepared by author	IV
pDSL_UGIH shTSC2-1	GATGTTTCAGCAGAATGTCC	Prepared by author	IV
pDSL_UGIH shTSC2-3	TGATGTCCGAGGAGAAAGG	Prepared by author	IV
pDSL_UGIH shPALS1-3	CTAGAGGTTGATTATCTTC	Prepared by author	IV
pDSL_UGIH shLATS1-1	CAGGAAATTCGAAACTCT	Prepared by author	IV
pDSL_UGIH shSTK3-3	GCAGGGATTAGCCTTAATAA	Prepared by author	IV
pLKO.1 shPALS1-2	GCAGGGATTAGCCTTAATAA	TRC library, #TRCN0000151036	IV
pLKO.1 shPALS1-3	GCTACTTTGTTAGGCTTGAAT	TRC library, #TRCN0000150865	IV
pLKO.1 shPALS1-4	CCCAGAGCTAAACACTGGAAT	TRC library, #TRCN0000156051	IV
pLKO.1 shLLGL2-5	CCGCGTCAAGTCCCTCAAGAA	TRC library, #TRCN0000116436	IV
pLKO.1 shCTNNB1-2	CGCATGGAAGAAATAGTTGAA	TRC library, #TRCN0000003844	IV
pLKO.1 shNUMB-1	CCTTGCAATTAGGCTAAAGAA	TRC library, #TRCN0000007223	IV
pLKO.1 shNUMB-2	GCAATCATTATGGCTATGTAT	TRC library, #TRCN0000007224	IV
pLKO.1 shNUMB-4	GCCATGTAGAAGTTGATGAAT	TRC library, #TRCN0000007226	IV
pLKO.1 shNUMB-5	GCATCACCTTTCCAAGGGAAT	TRC library, #TRCN0000007227	IV
pGIPZ shSCRIB-3	TCACTAGTTACAGTCTCGC	Open Biosystems, V2LHS_162	IV
pGIPZ shCDC42-1	TCTGTGATAATCCTCTTGC	Open Biosystems, V2LHS_261933	IV
pGIPZ shCDC42-2	TCAAATACATTCTTTAGGC	Open Biosystems, V2LMM_204452	IV
pGIPZ shRHOA-2	ATCTGTTACGGAGTAAAGC	Open Biosystems, V2LHS_237189	IV
pGIPZ shRHOA-3	AGTTATAAAGTAGTTACAG	Open Biosystems, V2LHS_132703	IV
pGIPZ shCADH1-2	TCAGTGTGGTGATTACGAC	Open Biosystems, V2LHS_14838	IV
pGIPZ shCADH1-3	TTAATCTCCAGCCAGTTGG	Open Biosystems, V2LHS_14837	IV
pGIPZ shCADH1-4	TACGTAGGGAACTCTCTC	Open Biosystems, V2LHS_243170	IV
pGIPZ shDLG1-1	AATATACAGTCATTGACCC	Open Biosystems, V2LHS_172916	IV
pGIPZ sh14-3-3-5	AGAACACAGAGAAGTTAAG	Open Biosystems, V2LHS_172083	IV
pGIPZ shNF2-2	ATTAACACGAAGCTTTGAG	Open Biosystems, V2LHS_201306	IV

Primary antibodies	Description	Source or reference	Used in
α 6-integrin subunit (CBL458)	mouse monoclonal	Cymbus Biotechnologies	I, IV
Actin (A4700)	mouse monoclonal	Sigma	I-IV
Active caspase-3 (9661)	rabbit polyclonal	Cell Signaling Technologies	I-IV
β -tubulin (ab6046)	rabbit polyclonal	AbCam	II-IV
Bak (ab-1)	mouse monoclonal	Calbiochem	II
Bax (6A7)	mouse monoclonal	Trevigen	II
Bcl-X _L (610211)	rabbit polyclonal	BD Transduction Laboratories	I, II
Bid (2002)	rabbit polyclonal	Cell Signaling Technologies	I, II
Bim (AAP-330E)	rabbit polyclonal	Stressgen	I
Caspase-8 (9746)	mouse monoclonal	Cell Signaling Technologies	I, II
CK14 (PRB-155P)	rabbit polyclonal	Covance	III
c-Myc (9E10; MMS-150P)	mouse monoclonal	Nordic Biosite	I, II
c-Myc (sc-788)	rabbit polyclonal	Santa Cruz	III
Cytochrome C (556433)	mouse monoclonal	BD Transduction Laboratories	I, II
Collagen I (ab34710)	rabbit polyclonal	AbCam	III
Collagen III (ab7778)	rabbit polyclonal	AbCam	III
Collagen IV (ab19808)	rabbit polyclonal	AbCam	III
Desmoplakin (ab16434)	mouse monoclonal	AbCam	III
E-cadherin (610182)	mouse monoclonal	BD Transduction Laboratories	I, III, IV
EpCAM (ab32392)	rabbit polyclonal	AbCam	III
GM130 (610823)	mouse monoclonal	BD Transduction Laboratories	I, III, IV
Hepsin (100022)	rabbit polyclonal	Cayman Chemicals	III
Ki-67 (NCL-Ki67p)	rabbit polyclonal	Novocastra Laboratories	I, IV
Ki-67 (M724901)	rat monoclonal	Dako	III
Laminin-332	rabbit polyclonal	Prof. Ismo Virtanen	III
Lkb1 (ab15095)	mouse monoclonal	AbCam	I, III, IV
Nidogen 1 & 2	rat monoclonal	Prof. Ismo Virtanen	III
Par-3 (07-330)	rabbit polyclonal	Upstate	III, IV
Par-6 (ab45394)	rabbit polyclonal	AbCam	IV
Phospho Histone-3 (9715)	rabbit polyclonal	Cell Signaling Technologies	III
Puma (ab9643)	rabbit polyclonal	Cell Signaling Technologies	II
ZO-1 (61-7300)	rabbit polyclonal	Zymed	III, IV

METHODS

The following section describes in detail the methods used by the author in this study.

Two-dimensional cell culture (I-IV)

Human mammary epithelial cell lines (MCF10A and HMEC-hT derived) were cultured in human mammary epithelial cell basal growth media MCDB 170 (US Biological) supplemented with bovine pituitary extract 70 µg/ml (BPE, Upstate), insulin 5 µl/ml, hydrocortisone 0.5 µg/ml, epidermal growth factor 5 ng/ml (EGF), transferrin 5 µg/ml, isoproterenol 10^{-5} M (all from Sigma) and antibiotics (amphotericin B 50 µg/ml and gentamicin 50 µg/ml, both from Sigma). Human lung fibroblast cell lines (MRC5-hT derived), Phoenix Ampho and 293fT cells were cultured in high-glucose containing Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS, Gibco), L-glutamine and antibiotics (penicillin and streptomycin, Gibco). All cells were stably maintained in humidified incubator at +37°C and 5% CO₂.

Three-dimensional organotypic cell culture (I,III,IV)

Basement membrane from Engelbreth-Holm-Swarm mouse sarcoma (Matrigeltm, Becton Dickinson) was prepared according to manufacturers instructions. To prepare on-top cultures, Matrigel was overlaid on 8-chamber slides and left to solidify for 15-20 minutes at +37°C. MCF10A cells grown on two-dimensional culture were trypsinized with 0.5% Trypsin-EDTA (Gibco) and collected and resuspended in assay media (MCDB 170 growth media with supplements containing 2% Matrigel). Cells were plated onto slides pre-coated with Matrigel 1500 cells/well. To prepare Matrigel embedded cultures, MMEC formed mammospheres were trypsinized with 0.05%Trypsin-EDTA for 5-10 minutes, centrifuged (900 rpm for 3 minutes) then suspended with liquid Matrigel and plated onto 8-chamber slides at approximately 1500 cells/well. To prepare Collagen I gel cultures, Collagen I gel (Rat tail Collagen I, Cultrex) and NaOH were mixed on ice with 10x phosphate buffered saline (PBS) to yield a final concentration of 3 mg/ml Collagen and 23 mM NaOH. The mixture was overlaid on 8-chamber slides and let to solidify in +37°C for 1 hour, after which approximately 1500 cells/well were plated onto slides. All three-dimensional MCF10A cultures were grown in MCDB 170 media with supplements and three-dimensional MMEC cultures in DMEM/F12 growth media lacking FCS. For both on-top and embedded cultures, media was replenished every fourth day.

Immunofluorescence of two-dimensional monolayer cultures (I-IV)

Samples were fixed with 4% paraformaldehyde (PFA) for 10 minutes at room temperature (RT). After fixation, cells were washed with PBS and permeabilized with 0.1% Triton-X in PBS for 5 minutes. Thereafter, samples were washed twice with PBS and non-specific binding sites were blocked with 1% bovine serum albumin (BSA,

Sigma) in PBS for 5 minutes. Next, samples were rinsed with PBS and incubated for 1 hour at RT with primary antibody (diluted in 1% BSA-PBS). Following incubation, samples were washed 3 times with PBS and then incubated for 30 minutes at RT with appropriate Alexa fluor dye (Molecular Probes) coupled anti-rabbit or anti-mouse secondary antibodies. Finally, samples were washed for 3 times with PBS followed by counterstaining of the cell nuclei with Hoechst33258 (Sigma). Slides were mounted using Immu-Mount (Thermo Scientific) mounting reagent. Images were acquired using Zeiss LSM Meta 510 confocal microscope (study III) or Axioplan 2 microscope (study I,II) equipped with mercury lamp and Zeiss AxioCam HRc color camera and Axiovision 4.4 software. The objectives used were Plan-Neofluar 20x (NA=0.5) and 40x (NA=0.75).

Immunofluorescence of three-dimensional organotypic cultures (I,III, IV)

The organotypic cultures were fixed with 2% PFA for 20 minutes at RT and thereafter washed with PBS. Epithelial structures were permeabilized with 0.25% Triton X-100 in PBS for 10 minutes at +4°C, and thereafter washed with PBS. The non-specific binding sites were blocked in immunofluorescence (IF) buffer (7.7 mM NaN₃, 0.1% BSA, 0.2% Triton X-100 (Sigma) and 0.05% Tween20 in PBS) supplemented with 10% normal goat serum (Gibco) for 1-2 hours. The primary antibody was incubated in the blocking solution overnight at +4°C. Following the incubation, structures were washed three times with IF buffer, 15 minutes each wash, and then incubated with appropriate Alexa Fluor secondary antibody diluted in blocking solution. After 40-50 minutes incubation at RT, the structures were washed with IF buffer as before and the nuclei were counterstained with Hoechst33258. Slides containing immunostained epithelial structures were mounted with Immu-Mount mounting reagent. Images of the structures were acquired using Zeiss Axiovert 200 microscope equipped with Zeiss Apotome and AxioCam MRm camera (study IV) and/or Zeiss LSM Meta 510 confocal microscope (studies I-IV) equipped with argon (488), helium-neon (543 and 633) and diode (405) lasers. The objectives used were Plan-Neofluar 40x DIC objective (NA=1.3, oil) and Plan-Neofluar 63x DIC (NA=1.25, oil).

Primary cell isolations and culture (III)

Primary MMECs were isolated from 8-14 weeks old virgin female mice. To isolate MMECs, mice were euthanized with CO₂ and cervical dislocation and thoracic and inguinal (#3, and #4) as well as #5 mammary glands were dissected. The lymph node in #4 glands was removed and the glands were finely chopped. Tissue was incubated with 0.01 mg of Collagenase A (Sigma) per 1 g of tissue in DMEM/F12 media (containing 2.5% FCS, 5 µg/ml insulin, 50 µg/ml gentamycin and 200 mM glutamine) with gentle shaking (120 rpm in environmental shaker) at +37°C for 2 hours or until solution turned milky orange. The resulting cell suspension was then first centrifuged at 1400 rpm for 10 minutes (Heraeus Multifuge 1.0) and consecutively pulse centrifuged 4-6 times at 1400 rpm to get a preparation free of cells other than MMEC

organoids. Next, organoids were trypsinized with 0.05% Trypsin-EDTA for 5-10 minutes to obtain smaller organoid units and drained through a 70 µm cell strainer (Beckton Dickinson). Thereafter cells were counted and plated on low adhesion plates (Nunc) for adenoviral or lentiviral infections in MMEC growth media (DMEM/F12 media containing insulin 5 µg/ml, hydrocortisone 1 µg/ml, mouse EGF 10 ng/ml, glutamine 200 mM, gentamycin 50 µg/ml and penicillin and streptomycin [all from Sigma] supplemented with 10% FCS).

Fat pad transplantations (III)

Fat pad transplantations were performed essentially as described previously (Deome *et al.*, 1959; Welm *et al.*, 2008). 3-week-old female FVB mice were anesthetized using 2-2.5% Isoflurane (Baxter) and the anterior part of #4 mammary gland containing rudimentary ductal epithelium, lymph node and bridge to #5 gland was surgically removed. MMECs at a 10⁵ cells/gland in 10 µl volume were injected to the remaining fat pad. The MMECs injected, which were infected either with shControl or shLkb1 (shLkb1-1 or shLkb1-2) construct, were injected into contralateral glands. The wound was sutured using wound clips (Autoclip Physicians kit, Clay Adams), which were removed 1 week after the operation. The mice received analgesic pre- and post-operatively (Temgesic 0.2 mg/kg and Rimadyl 5 mg/kg, respectively) and the recovery of the animals was followed 48 hours after surgery. In the experiments described in this study, fat pad transplanted mice were sacrificed as virgins 15 weeks after transplantation.

Calcium-switch assay (III)

MMECs were plated on glass coverslips and incubated overnight to form a confluent monolayer. The next day, 4 mM EGTA was added to chelate calcium for 30 minutes. After that, EGTA containing media was removed and replaced with MMEC growth media (containing calcium) and cells were incubated for an additional 2 hours and fixed and processed thereafter.

Immunohistochemistry (III)

All tissues embedded into paraffin were fixed in 4% PFA overnight and thereafter processed into paraffin blocks in Biomedicum Genomics Histolab. 5 µm thick sections of paraffin embedded tissues were deparaffinized and re-hydrated and incubated in antigen retrieval solution (Dako) overnight at +70°C followed by 1 hour antibody incubation (all other antibodies except Hepsin, where no antigen retrieval was performed and primary antibody was incubated overnight at +4°C) and standard avidin-biotin complex detection with 3,3-diaminobenzidine (Vector Laboratories) and counterstaining with hematoxylin (Thermo Scientific). TUNEL staining was performed with ApopTag in situ detection kit (Chemicon/Millipore) according to manufacturers instructions. For all paraffin embedded tissues, hematoxylin and eosin (H&E) staining were performed in Biomedicum Genomics Histolab. Imaging for representative

images as well as for analysis was performed with Leica DMB microscope with Olympus DP50 color camera.

Analysis of immunohistochemical stainings (III)

For analysis of Ki67 and TUNEL staining, the percentage of positive cells was quantitated from 40x fields using the Image J program (version 1.42q, National Institute of Health). 3-4 fields were analyzed per animal and >10 animals per genotype. For analysis of eosinophilic areas surrounding the mammary ducts, 20x images of H&E stained sections were used to measure the diameter of the eosinophilic area from the thickest point using the Image J program. 5-20 ducts were quantitated per animal and 10 animals per genotype were quantitated. Collagen IV immunostaining was quantitated from 20x and 40x images were taken from collagen IV immunostained sections from 5-20 ducts per animal. For analyses, the ducts were classified into 4 groups depending on the uniformity of collagen IV staining. The groups were uniform (staining evenly surrounding the duct), patchy (staining surrounding >50% of the duct), uneven (staining surrounding <50% of the duct) or absent (staining missing).

Wholemount stainings of mammary gland and analysis of branching morphogenesis (III)

#4 inguinal mammary glands were fixed in 4% PFA overnight and stained for several hours in carmine-alum staining solution (Carmine and Aluminum potassium sulfate, Sigma). After the desired color had developed, glands were washed with ethanol and mounted on glass coverslips and imaged using Leica MZ16 FA fluorescence stereomicroscope equipped with Leica DFC490 camera. The amount of branching morphogenesis was analyzed by quantitating the number of branch points in 40x fields. 3-4 fields were counted per animal with the aid of the Image J program.

Production of retroviruses and retroviral transductions (I, II)

Phoenix Amphi packaging cells were cultured in high-glucose containing D-MEM (Gibco) supplemented with 10% FCS, glutamine and antibiotics (penicillin and streptomycin). To produce amphotropic retrovirusparticles, a retroviral construct was introduced into the Phoenix Amphi cell line by transfecting 8 µg of DNA using JetPei transfection reagent (Polyplus) according to manufacturers instructions. The supernatants containing retroviruses were collected 48 hours post-transfection. Prior to retroviral infection, MCF10A or HMEC cells were plated at density of 1.5×10^5 cells/well of a 6-well dish. Transduced cells were selected in puromycin (2.5 µg/ml) or blasticidine (10 µg/ml) and surviving clones were pooled for assays.

Production of lentiviruses and lentiviral transductions (I-IV)

The VSVg pseudotyped lentivirusparticles were produced by transfecting 293FT cells with a three plasmid mixture consisting of the lentiviral expression or shRNA

construct, packaging construct CMVDelta8.9 and the VSVg encoding envelope construct phCMVg. For traditional high-titer lentivirus production, the supernatants containing lentiviruses were collected 72 hours post-transfection. To produce concentrated lentiviral particles, lentiviral particles were harvested 48-72 hours post-transfection and concentrated by ultracentrifugation 19 500 rpm for 140 minutes (Beckman Coulter Optima XL-80K Ultracentrifuge). The viral pellet was then resuspended into DMEM growth media to achieve approximately 185-fold concentration and left stand at +4°C for 2 hours. Viral titer was determined using p24 ELISA test measuring viral capsid protein p24 (Perkin Elmer, performed in Biomedicum Genomics). Lentiviral infections of 2D cultured human mammary epithelial cells were performed similarly as retroviral infections. Transduced MCF10A cells were selected in puromycin (2.5 µg/ml) or hygromycin (250 mg/ml) 72 hours post-transduction. In contrast, lentiviral infections to MMECs cultured on low-adhesion plates were performed in 24-well low-adhesion plates in <800 µl volume using multiplicity of infection 5. Cells were incubated with virus overnight. Transduced MMECs were washed with PBS and used 1 day post-transduction either in fat pad transplantations or placed directly to 3D culture.

Adenoviral transductions and analysis of Lkb1 knockdown (III)

Freshly isolated *Lkb1^{lox/lox}* or *Lkb1^{+/+}* control MMECs were counted and plated on low-adhesion plates at approximately 1-2 x 10⁶ cells/well, and AdenoCre or control AdenoGFP viruses (produced in Dr. T. Mäkelä's laboratory) were added to cells using multiplicity of infection 25. Cells were incubated with virus overnight. The next day, transduced MMECs were washed twice with MMEC growth media and left to incubate 96 hours in MMEC growth media supplemented with 10% FCS. Thereafter, cells were either lysed for western blotting or placed to 3D culture. For each isolated MMEC batch, *Lkb1* knockdown was analyzed by western blotting.

RNA isolation and quantitative real-time PCR (qPCR) (III)

RNA was isolated from MMECs using RNeasy isolation kit (Qiagen) and from tissues using AllPrep DNA/RNA kit (Qiagen) according to manufacturers instructions. For tissues, homogenization was done using ceramic beads (CK14, Bertin Technologies) and Precellys homogenizer (Bertin Technologies). cDNA synthesis was performed using DyNAmo cDNA synthesis kit (Finnzymes) and qPCR reactions using DyNAmo HS SYBR Green qPCR kit (Finnzymes) in AbiPrism 7500 Fast Real-Time PCR system (Applied Biosystems). Relative mRNA amounts were assayed by comparing PCR cycles to GAPDH using the $\Delta\Delta CT$ method (Livak and Schmittgen, 2001) and normalizing the samples to the control genotype.

Western blot analysis (I-IV) and subcellular fractionations (III) of cultured cells

Cells grown on 2D cell culture were lysed either in SDS lysis buffer (20% SDS in 250 mM Tris-HCl pH 6.8 – study I) or non-ionic Triton-X cell lysis buffer (10 mM Tris, pH 7.5, 130 mM NaCl, 1% Triton X-100, 10mM NaP_i and 10 mM Napp_i – study II). MMECs grown on low-adhesion plates were lysed in ELB lysis buffer (150 mM NaCl, 50 mM Hepes pH 7.4, 5 mM EDTA, 1% NP-40 – study III) supplemented with EDTA-free protease inhibitor cocktail (Roche). For detection of Hepsin, MMECs were lysed in PBS containing 1% Triton-X (study III), by letting the lysates stand 15 minutes on ice and subsequently centrifuging 13, 000 g for 15 minutes. From 3D acinar structures (study I), proteins were extracted with RIPA buffer (150 mM NaCl, 20 mM Tris pH 7.5, 0.1% sodium dodecyl sulfate, 1.0% sodium deoxycholate, 0.1% Triton X-100) for 15 minutes at +4°C. The lysates containing acinar structures and Matrigel were collected and pulled 5 times through a 25G needle and incubated on ice for 15 minutes. Lysates were then centrifuged (16 000 g for 20 minutes at +4°C). Subcellular fractionation was performed with the Pierce subcellular fractionation kit (Thermo Scientific) according to manufacturers instructions (study III). Protein concentration of all cell lysates was determined using Bio-Rad protein assay (Bio-Rad). 10-20 µg samples were denatured with 5x Laemmli sample buffer and separated in 10% SDS-PAGE gel, and subsequently transferred to nitrocellulose filters (Bio-Rad) followed by antigen detection with specific antibodies and ECL™ Detection Reagents (Amersham).

Western blot analysis of human and mouse tissue samples (III)

Mouse mammary glands and tumors and human breast cancer samples were lysed in mammary gland (MG)-lysis buffer (Zhu *et al.*, 2003) supplemented with EDTA-free protease inhibitor cocktail (Roche). An approximately 2 mm x 2 mm piece of snap frozen tissue was placed in tube containing MG-lysis buffer and ceramic beads (CK14) and homogenized using Precellys homogenizer (Bertin Technologies). Subsequently, samples were let stand on ice for 30 minutes and centrifuged at 13 000 g for 15 minutes. Protein concentrations and western blotting was performed as described above.

Image J analysis of 3D acini (IV)

MCF10A MycER cells were grown in 3D culture for 10 days and fixed and immunostained as described above. The structures were immunostained with E-cadherin antibody to visualize the borders of the acini and counterstained with Hoechst. Images of the stainings were acquired with the Zeiss Axiovert 200 microscope and Apotome system (Zeiss) equipped with MRr digital camera and Axiovision 4.4 program. The objective used was 20x Plan apochromat (NA=0.8). Digital images were opened in Image J software and converted to 16-bit format. Next, binary contrast enhancement (thresholding) was applied to the image by defining a grayscale cutoff point. The cutoff point was manually set by adjusting the threshold to accurately cover the acini by comparing them to the original image. Also, in the

thresholded image, acini that were slightly attached to each other were separated using the Pen tool. Finally, the thresholded and separated acini were measured for Area, Perimeter and Circularity (Shape descriptors). Multiple images were counted from each experiment to obtain measurements from at least 30 acini per genotype.

IV RESULTS AND DISCUSSION

5. Epithelial integrity suppresses oncogenic properties of c-Myc (I)

To explore how the epithelial integrity of mammary epithelial cells is able to modify the dual apoptosis and proliferation-promoting function of c-Myc, we took the advantage of using a three-dimensional (3D) organotypic cell culture model, in which cells are cultured on laminin rich basement membrane matrix (Debnath *et al.*, 2003a). In this model, the mammary epithelial cells undergo a defined set of morphogenic events leading to the formation of growth arrested and polarized structures termed acini, which preserve many features of mammary epithelium *in vivo* including cell polarity and cell-ECM contacts that are lacking in 2D culture (Debnath *et al.*, 2003a; Underwood *et al.*, 2006). It has been shown previously that introduction of other oncogenic lesions, such as Cyclin D1, HPV E7, ErbB2, Akt, or CSF-R1 to human non-transformed MCF10A cells leads to distorted morphology of the 3D acini, as well as deregulated proliferation and disrupted apoptosis control (Debnath *et al.*, 2002; Debnath *et al.*, 2003b; Muthuswamy *et al.*, 2001; Wrobel *et al.*, 2004). We thus introduced a conditionally active form of c-Myc, MycER, shown previously to mimic c-Myc functions (Eilers *et al.*, 1989; Littlewood *et al.*, 1995) into MCF10A cells and grew these cells in 3D culture (Matrigel) in the absence or presence of MycER activator 4-OHT. Acini formed in the presence of activated MycER exhibited increased apoptosis in immature day 5 3D structures and an increase in proliferation noted specifically in day 15 and 20 acinar structures (I, Figure 1). Furthermore, chronic c-Myc activation during acinar morphogenesis resulted in large and distorted acinar structures with filled lumen, corroborating previous results that oncogene activation in the developing 3D acini is able to disrupt acinar morphogenesis.

However, it was still unclear from previous experiments (Debnath *et al.*, 2002; Debnath *et al.*, 2003b; Muthuswamy *et al.*, 2001; Wrobel *et al.*, 2004), whether the pre-formed acinar structure was able to regulate oncogene-induced transformation, which prompted us to study the activation of MycER in mature acinar structures. Unexpectedly, activation of MycER in mature acinar structures failed to re-initiate cell cycle or induce apoptosis (I, Figure 2A-D). This was in contrast to pre-formed acini, thus, we next determined when acinar cells become refractory to c-Myc activation. Accordingly, if c-Myc was activated at days 1, 5 or 10, acinar cells were proliferative at day 20 whereas if c-Myc was activated at day 15 or 18, acinar cells remained Ki-67 negative (I, Figure 2E). These results suggest that the time when acinar cells become refractory to c-Myc activation coincides with acini undergoing quiescence and establishing the organized epithelial structure (Debnath *et al.*, 2002). Additionally, our results showed that activation of MycER in quiescent, growth factor deprived cells leads to rapid cell cycle re-entry (I, Figure S1). Thus we studied the alternative that not quiescence but intact epithelial integrity would inhibit c-Myc induced

proliferation. Essentially, collagen I matrix only weakly promotes acinar structure formation compared to Matrigel (Li *et al.*, 1987; Weaver *et al.*, 2002) and therefore, collagen I grown MCF10A exhibit relatively unorganized epithelial structure as demonstrated by diffuse $\alpha 6$ -integrin localization to the lateral sides of cells and lack of luminal space (I, Figure 3A). However, the majority of the collagen I matrix grown day 20 MCF10A structures were growth arrested, and intriguingly, in these settings activation of MycER resulted in cell cycle re-entry. In conclusion, these data suggest that epithelial integrity is critical for suppression of both the proliferative and apoptotic c-Myc function.

6. c-Myc-induced sensitization of mammary epithelial cells to apoptosis occurs by priming mitochondria independently of cell context (I, II)

In several cell types, activation of c-Myc induces apoptosis or apoptotic sensitization (reviewed in section 4), which is suggested to be an important cell-intrinsic tumor suppressor mechanism. Our results show that activation of c-Myc alone does not induce apoptosis in 2D cell culture, yet it sensitizes cells to a variety of stimuli including TRAIL, etoposide and cyclohexamide (I, Figure S1D and S5A). To study the molecular mechanisms leading to c-Myc-induced apoptotic sensitivity, we set out to study the apoptotic synergy between c-Myc and death receptor ligand TRAIL, which specifically kills cancer cells and is hence interesting regard to cancer therapeutics (Johnstone *et al.*, 2008). We demonstrate that TRAIL induces weak caspase-8 cleavage, but no visible apoptosis in MCF10A epithelial or MRC5 fibroblast cell lines, but in the presence of both activated c-Myc and TRAIL, wide-spread apoptosis was observed (II, Figure 1-2). Furthermore, we observed activation of pro-apoptotic proteins Bak and Bax, which is indicative of mitochondrial apoptosis pathway activation. Previous studies have implied a role for c-Myc in the regulation of the mitochondrial apoptosis pathway (Hemann *et al.*, 2005; Maclean *et al.*, 2003; Mitchell *et al.*, 2000), through modulation of the pro- and anti-apoptotic Bcl-2 family members Bax and Bim by upregulation, and Bcl-X_L by downregulation. However, no changes in the levels of these proteins were observed (II, Figure 7A), instead, a temporary upregulation of Bak was seen. Furthermore, intense mitochondrial immunofluorescence staining of active Bak was seen after both activation of c-Myc and addition of TRAIL, but unexpectedly, a weak staining pattern was seen when c-Myc was activated alone (II, Figure 7B). Thus, c-Myc induces “pre-activation” of Bak proteins without induction of apoptosis, which could be indicative of priming of the mitochondria for apoptosis. It has been previously suggested that c-Myc sensitizes cells to TRAIL-induced apoptosis by inhibiting FLIP and thus active caspase-8 generation at DISC (Ricci *et al.*, 2007), whereas our results propose a model where TRAIL induces activation of the upstream regulators caspase-8 and Bid while c-Myc induces the activation of Bax and Bak. Our results thus imply that c-Myc amplifies weak caspase-8-Bid activation by TRAIL through the transient upregulation and pre-activation of Bak, which further leads to Bax activation and cytochrome c release from the mitochondria (II, Figures 7-8). Thus

it is interesting to speculate whether c-Myc deregulation contributes to the specificity of tumor cells to TRAIL-induced apoptosis and whether it could thus be used as a therapeutic target for killing cancer cells.

Even though the sensitivity to c-Myc-induced apoptosis was markedly different in immature and mature 3D acinar structures, c-Myc was able to induce sensitization to TRAIL-induced apoptosis also in the mature acini (I, Figure 5 and S5E). Moreover, c-Myc and TRAIL-induced apoptotic synergy was inhibited in both 2D and 3D cell culture by overexpression of anti-apoptotic Bcl-X_L and shRNA-mediated silencing of caspase-8, Bim or Bid (I, Figure 5C; II Figure 2-3). Thus these data suggest that c-Myc induced apoptotic sensitivity is modulated by Bcl-2 family members and proceeds via the mitochondrial route independently of cell context and microenvironment. Also, previous studies have shown that organized epithelial structure is able to suppress apoptosis induced by a variety of stimuli (Boudreau *et al.*, 1996; Weaver *et al.*, 2002). Indeed, in unorganized acini, the combination of c-Myc and TRAIL led to apoptotic hypersensitivity, further suggesting that unorganized structures are more vulnerable undergo apoptosis. Furthermore, our results indicate that while the molecular mechanism of c-Myc-induced apoptotic sensitization is context independent, epithelial integrity can act as a strong modulator of cell death decisions.

7. Lkb1 controls epithelial integrity of 3D mammary epithelial acini and synergizes with c-Myc in induction of proliferation (I, III)

Previous studies in *Drosophila* epithelial cells as well as in avian embryos (Brumby and Richardson, 2003; Dolberg and Bissell, 1984; Pagliarini and Xu, 2003) have suggested that organization of cells into an intact tissue structure can have an important effect on the capability of oncogenes to induce cell proliferation. In accordance, our results suggested, that intact epithelial structure affects the ability of c-Myc to induce cell proliferation. Hence, we decided to downregulate tumor suppressor LKB1, the mammalian homolog of the polarity protein PAR-4, which has been implicated in regulation of various aspects of epithelial integrity (Amin *et al.*, 2009; Baas *et al.*, 2004; Hezel *et al.*, 2008). The MCF10A MycER acini stably expressing Lkb1 shRNA exhibited a distorted shape, mislocalization of the apical polarity marker GM130 to the lateral sides of cells as well as a lack of luminal space (I, Figure 4A-B), all indicative of loss of epithelial integrity. Thus these results verify previous studies demonstrating that loss of Lkb1 leads to loss of apico-basal polarity and rearrangement of epithelial junctions (Amin *et al.*, 2009; Hezel *et al.*, 2008; Martin and St Johnston, 2003). Lkb1 has previously been reported to control cell cycle progression (Tiainen *et al.*, 2002) and accordingly, we observed that Lkb1 lacking acini grew larger in size and exhibited a slightly prolonged proliferation period before undergoing quiescence. Not surprisingly, activation of c-Myc in Lkb1 deficient acinar cells from the beginning of acinar morphogenesis led to the formation of large, misshapen structures exhibiting widespread proliferation and lack of normal epithelial structure. However, when MycER was activated in mature, Lkb1 lacking

acini, the acinar cells re-entered the cell cycle whereas control cells remained quiescent (I, Figure 4D), implying that Lkb1, via regulation of epithelial integrity, has an important role in suppressing cell cycle progression induced by c-Myc.

Similar results were observed in mouse mammary epithelial cells (MMECs) lacking Lkb1. To study the loss of Lkb1 in the mouse mammary gland, we used isolated MMECs from female mice carrying conditional *Lkb1^{lox/lox}* alleles (Bardeesy *et al.*, 2002), and infected them with Adenoviral Cre recombinase leading to nearly complete loss of Lkb1 (III, Figure 1A). In 3D culture, Lkb1 lacking MMECs formed acinar structures that were clearly larger and deformed in morphology when compared to control acini (+/+ Adeno Cre infected and uninfected). Furthermore, loss of Lkb1 from MMECs lead to cell polarity defects as shown by loss of apical polarity markers Par-3 and GM130 as well as lack of luminal space. We also observed lateralization of the tight junction (TJ) marker ZO-1 into the basolateral compartment, co-localizing with adherens junction (AJ) marker E-cadherin, thus suggesting disrupted organization of junctional complexes in Lkb1 lacking acini (III, Figures 1B-D). These observations were confirmed with transmission electron microscopy (TEM), where lengthening of the physical TJ complex was seen (III, Figure 1E-F, S1F). Intriguingly, in addition to these defects, we also observed spontaneous branching morphogenesis as well defects in the basement membrane (BM) of the Lkb1 lacking acini (III, Figure 2). Hence, these results further validate the role of Lkb1 in control of epithelial integrity in mammary epithelial cells.

To explore whether Lkb1 loss and active c-Myc also cooperate in the induction of cell proliferation in MMECs, we isolated cells from mice carrying both *Lkb1^{lox/lox}* and Wap-Myc alleles (Sandgren *et al.*, 1995). The Wap promoter-driven transgenes have previously been demonstrated to be activated *in vitro* by addition of lactogenic hormones (Chen and Bissell, 1989). However, we found that Wap-Myc was biologically active *in vitro* in isolated MMECs without lactogenic hormones (III, Figure S5), likely due to deregulation of Wap promoter control by active c-Myc (Schoenenberger *et al.*, 1988). Corroborating our findings in MCF10A cells, MMECs with deregulated c-Myc and loss of Lkb1 exhibited prominently increased cell proliferation and marked changes in acinar structure. The structures that developed were larger compared to acini formed in the presence of Wap-Myc or Lkb1 loss alone and additionally exhibited a complex phenotype reminiscent of branching but also of acinar aggregation. They were similarly lacking luminal space, apical organization of TJ marker ZO-1 and almost completely lacked BM collagen IV staining (III, Figure 5B-C), altogether suggesting a wide-spread lack of epithelial organization and proliferation control. In this system, we could not test whether c-Myc activation in the preformed acini deficient in Lkb1 could induce ectopic proliferation, however, our results suggest that loss of Lkb1 dramatically increases the proliferation-inducing capability of c-Myc also in MMECs.

8. Lkb1 controls epithelial integrity and branching morphogenesis *in vivo* in the mouse mammary gland (III)

Because our studies strongly suggested Lkb1 is important in the regulation of epithelial architecture of mammary epithelial cells, we next explored whether Lkb1 controls epithelial structure also *in vivo*. Therefore, we used transgenic and mammary fat pad transplantation based approaches to delete Lkb1 from the mammary epithelium. Firstly, we crossed *Lkb1^{lox/lox}* mice with Wap-Cre mice (Wagner *et al.*, 1997) to create animals with tissue specific deletion of Lkb1 from the mammary epithelium. The deletion of Lkb1 from the mammary epithelium was confirmed by genomic PCR verifying the occurrence of recombination, and by qPCR analysis of Lkb1 mRNA (III, Figure S3A-B). Interestingly, we detected both recombination of the Lkb1 allele and loss of Lkb1 mRNA from the virgin mammary epithelium, most likely due to minor Cre activity during estrous cycles (Robinson *et al.*, 1995). Indeed, in the virgin mammary gland, increased lateral side branching of the Lkb1 lacking epithelium was observed (III, Figure 3A), yet the ultrastructure of the glands were similar to control glands as demonstrated by H&E, CK14 and EpCAM stainings. Interestingly, similar lateralization of TJs in the Lkb1 lacking ducts was observed as in 3D culture *in vitro* and mild disorganization of the ductal epithelium illustrated by staining with apical markers occludin and ZO-1 (III, Figure 3F). These results suggest that while loss of Lkb1 induces cell polarity defects in the mammary epithelium, it does not result in a widespread disarray of the tissue. In accordance, a recent study showing loss of Lkb1 from the mouse mammary gland using the Blg-Cre promoter does not report gross alterations of Lkb1 lacking glands (McCarthy *et al.*, 2009), even though this study did not exhibit detailed analysis of mammary gland ultrastructure nor polarity markers.

Comparable results were obtained from the fat pad transplantation assays. MMECs from wild type donor mice were lentivirally transduced to express Lkb1 shRNA, transplanted into cleared fat pads of recipient virgin mice and analyzed 14 weeks after transplantation. In the resulting mammary epithelium, a significant decrease in Lkb1 mRNA levels was observed and similarly to the transgenic animals, increased side branching accompanied with markedly normal ultrastructure was observed. However, both transgenic and fat pad transplanted Lkb1 lacking epithelium showed increased eosinophilic staining and thickening surrounding the mammary ducts, which coincided with immunostaining of interstitial collagens I and III (III, Figure 3B-E). Furthermore, in the Lkb1 lacking epithelium, the staining of BM collagen IV was observed in both transgenic and fat pad transplanted animals to be discontinuous. Thus our results imply that loss of Lkb1 not only leads to defects in epithelial integrity, but also to changes in the microenvironment surrounding the epithelium. Interestingly, previous studies have shown that loss of Lkb1 alters the stromal component of the gastro-intestinal tract (Udd *et al.*, 2010) and tumor stroma in a lung cancer model (Gao *et al.*, 2010b). Moreover, loss of Lkb1 exclusively from the stromal smooth muscle cells can lead to polyp formation (Katajisto *et al.*, 2008). Taken

together, it appears that loss of Lkb1 affects the signaling between stromal and epithelial cells, thereby leading to altered growth control of the epithelium.

Most interestingly, our results suggest that Lkb1 plays a role in mammary branching morphogenesis. A previous study has also implicated apico-basal polarity protein Par-3 in branching morphogenesis. The study shows that loss of polarity protein Par-3 from mammary epithelial cells results in inhibition of ductal elongation and branching morphogenesis (McCaffrey and Macara, 2009). The authors speculate that this is due to incorrect positioning of the apical aPKC as well as differentiation defects in the terminal end buds (TEBs) cell population, leading to inability of the TEBs to bifurcate and the ducts to elongate. Furthermore, Par-3 and Par-6/aPKC proteins have been implicated in the orientation of the mitotic spindle (Durgan *et al.*, 2011; Hao *et al.*, 2010), which if defective could in mammary epithelial cells lead to disorientation of the cells within the developing epithelium. In the case of Lkb1, however, it appears that the cell polarity defects in Lkb1 lacking cells instead lead to epithelium sensitive for hyperactivated branching morphogenesis. Thus it could be hypothesized that the relatively mild polarity defects in Lkb1 deficient glands lead to increased branching, whereas gross defects, such as in the case of Par-3 deficiency, lead to the opposite phenotype. Indeed, it has been shown that activation of mutant Ras in a dose-dependent manner leads to hyperplasia and inhibition of mammary ductal morphogenesis (Sarkisian *et al.*, 2007).

Furthermore, it is tempting to speculate that Lkb1 could contribute to branching morphogenesis by modulating the stroma of mammary epithelial ducts. Our results show that loss of Lkb1 leads to an increase in the interstitial collagens I and III surrounding the mammary ducts. Interestingly, in a lung cancer model, loss of Lkb1 has been shown to contribute to cancer progression by increasing lysyl oxidase activation, which in turn leads to increased deposition of collagen and enhanced proliferation (Gao *et al.*, 2010b). It has also been demonstrated that collagen I can be found from the ECM surrounding TEBs (Ingman *et al.*, 2006) and moreover, that collagens and crosslinking of collagen increases the compliance of the ECM, which can in turn lead to increased cell motility and malignant phenotype of mammary epithelial cells (Levental *et al.*, 2009). Thus these data suggest that Lkb1 deficiency-induced increases in the interstitial collagens could concomitantly increase the propensity of mammary epithelial ducts to branch.

Lkb1 could also affect branching morphogenesis by regulating TGF- β 1 signaling. TGF- β 1 appears to have a dual role in regulating branching morphogenesis, as both overexpression and loss of TGF- β 1 have been demonstrated to inhibit branching morphogenesis (Daniel *et al.*, 1989). However, collectively the results suggest that TGF- β 1 signaling is increased at TEBs and activated elsewhere in the ducts to inhibit excess lateral branch formation (Silberstein *et al.*, 1992). Moreover, mouse embryonic fibroblasts lacking Lkb1 were shown to exhibit lower levels of TGF- β 1, due to a decrease in TGF- β 1 mRNA production and TGF- β 1 downstream effector Smad activity

(Katajisto *et al.*, 2008; Vaahtomeri *et al.*, 2008), furthermore, deficient stromal TGF- β 1 signaling was suggested to be a possible mechanism leading to epithelial proliferation during gastrointestinal polyp formation (Katajisto *et al.*, 2008). Our 3D *in vitro* screen revealed that TGF- β 1 was able to inhibit Lkb1 loss-induced spontaneous branching *in vitro*. Taken together, Lkb1 deficiency could lead to increase in lateral side branching due to defective TGF- β 1 signaling in the ducts.

Finally, Lkb1 loss was observed to lead to BM deterioration both *in vitro* and *in vivo*. It has been demonstrated previously that an increase in Stromelysin (MMP-3), which has been shown to degrade BM, results in increased side branching (Sympson *et al.*, 1994). Interestingly, our results imply that serine-protease Hepsin, which has been shown to cleave laminin-332 *in vitro* and when overexpressed leads to BM deterioration *in vivo* (Klezovitch *et al.*, 2004; Tripathi *et al.*, 2008), is a downstream target of Lkb1 in the regulation of BM in mammary epithelial cells (Discussed in more detail in 9). We found in our studies that Hepsin is deregulated in MMECs lacking Lkb1 and furthermore, that downregulation of Hepsin in Lkb1 deficient MMECS inhibits spontaneous branching and leads to restoration of intact BM (III, Figure 6D-H). Furthermore, hepatocyte growth factor (HGF), a recently demonstrated target of Hepsin (Owen *et al.*, 2010), has also been implicated in mammary branching morphogenesis (Yant *et al.*, 1998). Therefore, it is plausible that loss of Lkb1 could lead to Hepsin deregulation also *in vivo* and thus to enhanced branching morphogenesis via mechanisms involving BM deterioration or HGF activation.

9. Loss of Lkb1 cooperates with c-Myc in mammary tumorigenesis (III)

As our studies demonstrated that loss of Lkb1 sensitizes cells to proliferation and malignant growth induced by oncogenic c-Myc *in vitro*, we explored whether loss of Lkb1 *in vivo* would accelerate c-Myc-induced tumorigenesis. Thus we created triple-transgenic mice by crossing together animals carrying Wap-Myc, Wap-Cre and *Lkb1*^{lox/lox} alleles (hereafter Myc/Lkb1-), allowing us to simultaneously study the activation of c-Myc and Lkb1 deletion from mouse mammary epithelium. Previous studies have shown that female mice overexpressing c-Myc in mammary epithelial cells develop mammary carcinomas in multiparous state with a latency varying from 4 to 6 months (D'Cruz *et al.*, 2001; Sandgren *et al.*, 1995; Schoenenberger *et al.*, 1988). We observed similar latency (6 months) after two consecutive pregnancies in mice expressing Wap-Myc (or in Wap-Myc;Wap-Cre animals), yet virgin mice already exhibited increased proliferation and apoptosis. In contrast, the Myc/Lkb1- mice exhibited dramatically shortened latency of tumor formation (2.5 months), as they developed tumors already during or before the first pregnancy (III, Figure 4). Furthermore, whereas Wap-Myc mice developed approximately 2.5 focal or multifocal tumors, Myc/Lkb1- mice each developed 10 tumors. Also, in the Myc/Lkb1- animals the tumors were diffuse and >6 times the volume, consisting essentially the whole gland. The phenotype of mice lacking only one Lkb1 allele was similar to Wap-Myc mice, with higher tumor multiplicity (mean 6.5). Intriguingly, also 65% of male

Myc/Lkb1- mice developed mammary tumors with lower multiplicity (mean of 3.6 tumors). Taken together, loss of Lkb1 dramatically synergizes with c-Myc in mammary tumorigenesis.

The histopathological analysis of tumors, revealed that the Wap-Myc and Myc/Lkb1- tumors were markedly similar (III, Figure 4D and Table S6). Although all the tumors were cytologically of high grade, half of the Myc/Lkb1- tumors were classified as adenocarcinomas and half as mammary intraepithelial neoplasias, mainly because they were due to a diffuse tumor type lacking observable invasion. However, all the Myc/Lkb1- tumors had a distinct morphology as they exhibited large, unorganized and coalesced epithelial ducts as well as irregular, surrounding BM. Similar coalesced structures were also present in the rare, non-tumor areas of Myc/Lkb1- animals (III, Figure 5A). Hence, the fast tumorigenesis in Myc/Lkb1- animals and the distinct morphology of the glands including unorganized epithelial structures and deterioration of BM suggested that Lkb1 loss-induced epithelial integrity disruption could be important in the formation of Myc/Lkb1- tumors.

Furthermore, we did not observe inhibition of apoptosis, which has been shown to accelerate c-Myc-induced tumorigenesis in different mouse models (Amundadottir *et al.*, 1996; Zhan *et al.*, 2008). For instance, loss of the polarity protein Scribble from mammary epithelial cells leads to disruption of epithelial integrity and an increase in c-Myc-induced tumorigenesis by means of inhibiting c-Myc-induced apoptosis. However, the Myc tumors lacking Scribble were shown to be well-differentiated and low-grade (Zhan *et al.*, 2008) suggesting, in this case, that the tumorigenic cooperation of Myc with loss of epithelial architecture occurs by a mechanism different from Lkb1 loss. Moreover, our results imply that c-Myc-induced apoptosis is not the only factor limiting c-Myc-induced tumor growth. Also, inhibition of proliferation by tissue structure might similarly lead to slow tumorigenesis by c-Myc. For instance, it has been shown that overexpression of c-Myc in developing hepatocytes leads to rapid onset of neoplasia, but if c-Myc is activated in adult hepatocytes within differentiated tissue, neoplasias develop only after long latency (Beer *et al.*, 2004). Similarly, activation of c-Myc in multiple tissues under the ubiquitous Rosa26 promoter led to increased cell proliferation of many but not all tissues (Murphy *et al.*, 2008).

We thus hypothesized that the strong synergy between c-Myc activation and Lkb1 loss in tumorigenesis is attributable to Lkb1 loss-induced defective epithelial integrity and BM, and simultaneous c-Myc-induced proliferation of the alveolar cells. Furthermore, we hypothesized that pathways linked to branching morphogenesis might also play a role in the synergy between c-Myc activation and Lkb1 loss. Therefore, we next targeted pathways linked to epithelial integrity and branching morphogenesis in order to rescue Lkb1 loss-induced spontaneous branching and Myc/Lkb1- induced branching/aggregation phenotypes (III, Figure 5D-F). Our results revealed that while TGF- β 1 was able to inhibit loss of Lkb1 induced spontaneous branching, the

Myc/Lkb1- induced branching/aggregation was only inhibited by trypsin-like serine protease inhibitor Compound 10a.

Therefore, we focused on studying serine-protease Hepsin, an interesting candidate shown previously to be deregulated in prostate and breast cancer and to cooperate with c-Myc in induction of prostate carcinogenesis (Klezovitch *et al.*, 2004; Nandana *et al.*, 2010; Xing *et al.*, 2011), interestingly, Hepsin has been shown to induce degradation of BM by directly cleaving laminin-332 (Tripathi *et al.*, 2008). Surprisingly, we found that in Lkb1 deficient MMEC acini, Hepsin was found to be mislocalized from cell-cell borders to the cytosol (III, Figure 6A). Hepsin has been demonstrated to reside in desmosomes (Miao *et al.*, 2008), and likewise, we show that it partially co-localizes with desmosomal protein desmoplakin. Furthermore, both proteins are concomitantly localized to cytosol upon Lkb1 loss or calcium removal-induced loss of epithelial junctions, which suggests, that disturbances in epithelial cell junctions leads to Hepsin mislocalization. We also demonstrate that overexpression of Hepsin leads to similar disruption of epithelial integrity as exhibited by loss of Lkb1, as demonstrated by lack of luminal space, mislocalization of apical polarity marker ZO-1 and discontinuities in BM marker Nidogen in acini overexpressing Hepsin. Furthermore, downregulation of Hepsin by lentiviral shRNA leads to partial inhibition of Lkb1 loss-induced spontaneous branching and Myc/Lkb1- induced branching/aggregation as well as to re-establishment of BM (III, Figure 6D-H). Also, Hepsin expression was observed to be negative in the majority of Wap-Myc tumors (75%) by immunohistochemical staining, while in contrast, 65% of the Myc/Lkb1-tumors exhibited intense Hepsin immunostaining (III, Figure 7A). Also, Hepsin protein was observed exclusively in Myc/Lkb1- tumors by western blotting (III, Figure 7B). These results thus propose that deregulation of Hepsin, involving mislocalization and overexpression, mediates Lkb1-induced epithelial disorganization, spontaneous branching and BM deterioration.

Finally, we studied the expression of Lkb1 and Hepsin in a set of 57 unselected human breast cancer samples. Lkb1 mutations are known to be rare in sporadic breast cancers yet diminished expression has been reported (Fenton *et al.*, 2006; Shen *et al.*, 2002). Hence, we determined the protein levels of Lkb1 in these samples by western blotting and Hepsin levels by immunohistochemistry. Interestingly, 26% of the samples exhibited diminished Lkb1 protein levels comparable to the MCF10A cell line, which exhibited relatively low Lkb1 levels (data not shown). Moreover, out of these samples, 47% demonstrate strong Hepsin immunostaining yet surprisingly none of the samples were Hepsin negative (III, Figure 7C-E). Thus our results suggest that diminished Lkb1 levels associate with deregulation of Hepsin in breast cancer. In conclusion, these results show that loss of *Lkb1* is a strong collaborator with c-Myc in tumorigenesis. Furthermore, they propose that Lkb1 loss-induced disruption of the mammary epithelial integrity, mediated by Hepsin, is causing the strong synergy with c-Myc. Thus it is interesting to speculate whether these defects, including cell polarity

disruption, increased interstitial collagen production, increased branching and BM deterioration seen in mammary epithelium lacking *Lkb1*, have in combination a stronger effect on c-Myc-induced tumorigenesis as they would as single factors.

10. Screen for mammalian epithelial integrity regulating factors reveals critical modulators of both apoptosis and proliferation (IV)

Our previous results suggested that epithelial integrity of mammary epithelial cells critically modulates proliferation induced by oncogenes. Furthermore, in *Drosophila*, various genes known to regulate cell polarity as well as other aspects of epithelial integrity are also required for cell proliferation control (Brumby and Richardson, 2005) and mutations in these genes lead to disruption of epithelial integrity and eventual tumor growth. We thus explored whether human orthologs of these *Drosophila* genes regulate epithelial integrity and cell proliferation of mammary epithelial cells, and additionally, whether these genes have tumor suppressor properties.

We compiled a list using *Drosophila* Interactive Fly database (47th Edition; Brody, 1999) and identified genes related to tumor suppression, epithelial junctions, apico-basal cell polarity, tissue polarity and asymmetric cell division. The list included cell polarity and adhesion regulators, *Drosophila* hTSGs and nTSGs, and other signaling molecules. Next we searched for the human orthologs of these genes using Ensembl database (ensembl.org) to yield a list of potential human epithelial integrity regulators (hEIRs). Also, as TJs have a different function and localization in *Drosophila* and mammalian epithelial cells, we added to the list TJ related proteins to yield a final list of 77 genes (IV, Figure 1). To assess the tumor suppressor potential of the hEIR genes, we created an algorithm that compiles data from global databases (ONCOMINE, COSMIC, Tumorscape and The Cancer Genome Atlas) and records the somatic mutation frequency, loss of expression and copy number of genes and chromosomal rearrangements. This algorithm was applied to all human genes to rank them according to their tumor suppressor potential. We observed that 20 out of the 77 hEIR genes were among the genes ranking highest in tumor suppressor properties (IV, Figure 1B-C).

Next we assessed the effect of knocking down hEIR genes on the formation and maintenance of mammary epithelial 3D structures. To create a collection of validated shRNAs, we collected 2-5 shRNAs against all the hEIR genes (IV, Tables 2-4) and tested the knockdown efficacy of these constructs by qPCR or western blotting. In total 52 constructs (24% of all) produced a knockdown, which is similar to commercially available shRNA libraries. Next, to screen for defects in epithelial integrity, the MCF10A MycER cells were individually infected with each of the 52 validated shRNAs, and grown in 3D culture for 10 days in the presence or absence of MycER activation. Subsequently, acini were immunostained with E-cadherin and

analyzed for size (area and perimeter) and symmetry (circularity), both important aspects of epithelial integrity. Interestingly, 19 shRNAs against 16 different genes exhibited changes in the epithelial structure (IV, Figure 2 and Table 1). The genes leading to changes in acinar size included for instance *DVL3*, *PALS1*, *NUMB* and *LATS1*. Also, only 6 shRNA constructs against resulted in a decrease in acinar symmetry (IV, Table 1). Intriguingly, the *DVL3-B* and *PALS1-B* shRNA constructs, which lead to the most prominent size increase in the MCF10A acini, also lead to a significant increase in acinar proliferation at day 18. In conclusion, our screen revealed that many of the human orthologs of *Drosophila* epithelial integrity regulating genes also have a key role in the regulation of epithelial integrity in human mammary epithelial cells. Additionally, it seems that many of these genes lead to disruption of growth control during acinar morphogenesis and furthermore, that severe changes in acinar morphology are often accompanied by size increase. Thus, these data suggest that genes operating in an nTSG-like fashion can also be found in mammalian cells.

Next we wanted to explore the transforming cooperation of hEIR downregulation with oncogene c-Myc. Moreover, ours as well as others' results demonstrate that disruption of epithelial integrity can enhance the MCF10A acinar transformation induced by c-Myc (I, III). Therefore we tested the effect of chronic c-Myc activation starting at day 1 in the 52 shRNAs with validated knockdown (IV, Figure 3). Our previous results show that c-Myc activation leads to an increase in acinar size and misshapen acini (I), yet nevertheless 21 constructs against 15 individual genes (IV, listed in Table 1) produced alterations in acinar size and symmetry compared to control shRNA. Furthermore, loss of expression of polarity gene *PALS1* or actin binding tropomodulin 3 *TMOD3* exhibited both simultaneous increase in acinar size and decrease in symmetry and additionally increased proliferation at day 18 (IV, Figure S4). Interestingly, our screen revealed that downregulation of altogether 15 individual genes (IV, Table 1) to decreased acinar size in combination with MycER activation. Two of these constructs targeting important regulators of cytoskeleton and cell polarity, led not only to decreased acinar size but also to a significant decrease in acinar symmetry. Closer inspection of the acini by immunostaining of active caspase-3 revealed enhanced apoptosis in these acini (IV, Figure 3E-F), implying a strong synthetic lethal interaction between loss of these gene functions and c-Myc activation. However, the synthetic lethality was only observed in 3D culture, suggesting that this cooperation with c-Myc in induction of apoptosis is dependent on cell context.

Finally, we assessed the effect of acute activation of MycER in mature, growth-arrested acini expressing each of the shRNAs affecting epithelial integrity. As our previous results indicate (I) that disruption of epithelial integrity by loss of polarity protein Lkb1 leads to restoration of c-Myc's ability to induce cell cycle progression, we hypothesized that other epithelial integrity regulating genes might also function in a similar fashion. Most of the shRNAs tested induced cell proliferation already independently of c-Myc activation, and thus did not exhibit marked increase in

proliferation when c-Myc was activated. Surprisingly, only 5 shRNAs cooperated with acute c-Myc activation in the induction of proliferation in 3D structure. However, with 4 of the constructs, the proliferation was slightly increased without c-Myc activation, but was further increased when c-Myc was activated (IV, Figure S4). Interestingly, only one shRNA construct was able to cooperate in the induction of proliferation with c-Myc in a fashion similar to shLkb1 (IV, Figure 4). The mechanisms behind the interaction between this polarity gene and c-Myc activation are currently under investigation and are discussed in detail in IV.

Taken together, the data presented here underline the importance of epithelial integrity in controlling the homeostasis of epithelium. Furthermore, these studies demonstrate that intact epithelial structure controls normal and unscheduled cell proliferation *in vitro* and *in vivo*, and thereby suggest that intact epithelial structure can act as a mechanism resisting oncogene-induced proliferation. Moreover, this study has revealed Lkb1 as a major protein responsible for formation of intact epithelial structure and suggests that other proteins are also involved. Thus the present study brings insight into new genes regulating epithelial integrity of mammalian cells. Furthermore, this study shows that, similar to *Drosophila* (Bilder, 2004), loss of certain epithelial integrity regulating genes leads to deterioration of the epithelial structure without affecting cell proliferation, while loss of other epithelial structure regulating genes can affect both epithelial integrity and proliferation. Importantly, certain epithelial integrity regulators, when lost, can cooperate in proliferation induction by c-Myc oncogene. Therefore, as cancer is a disease of uncontrolled cell proliferation and loss of epithelial integrity is a hallmark of advanced tumors (Hanahan and Weinberg, 2011; Tervonen *et al.*, 2011), it is interesting to speculate whether epithelial integrity compromising lesions could be found from benign lesions or low-grade tumors. Furthermore, if such lesions are present already in early tumors, they could be potentially used to predict disease outcome, as for instance is the case with E-cadherin (Mastracci *et al.*, 2005; Rakha *et al.*, 2005). In addition, while the studies presented here explored the effect of epithelial integrity on the functions of c-Myc, it could be anticipated that the function of other oncogenes is also modulated by epithelial integrity. For example, in *Drosophila* disruption of epithelial integrity cooperates with Ras or Notch oncogenes in induction of tumor growth and metastatic behavior (Brumby and Richardson, 2003; Pagliarini and Xu, 2003), and thus it can be hypothesized that epithelial integrity also presents a proliferation barrier for other oncogenes in mammalian cells. Future studies will be required to fully understand the role of epithelial integrity in the regulation of cell growth and in proliferation, yet this data brings new insight into genes relevant for initiation of tumorigenesis as well as for conversion of benign lesions into invasive, malignant disease.

V CONCLUSIONS AND FUTURE PERSPECTIVES

The studies presented here provide compelling evidence that epithelial integrity, formed by the interplay between cell-cell and cell-ECM adhesions and cell polarity, functions as a tumor suppressor mechanism by inhibiting oncogene-induced cell proliferation. The concept of epithelial integrity as a tumor suppressor, while previously recognized in *Drosophila* (Bilder *et al.*, 2000), has not been widely studied in mammalian systems. Therefore, the major and most novel finding of this study is that oncogene c-Myc-induced cell proliferation and tumor progression are inhibited by intact epithelial integrity (I, III).

The data presented herein also suggests that Lkb1, a polarity protein implicated in human tumor suppression, is essential in regulation of epithelial integrity in the mammary epithelium (I, III). This is demonstrated by wide spread disturbance in epithelial integrity, including loss of apico-basal polarity, BM deterioration and increased side branching. The multiple defects inflicted by loss of Lkb1 could reflect the fact that Lkb1 controls several downstream pathways by 14 downstream kinases (Lizcano *et al.*, 2004), many of which have been implicated in regulation of different aspects of epithelial integrity. However, while the precise mechanisms by which Lkb1 regulates epithelial integrity warrant further investigation, these studies reveal a link between Lkb1 loss and deregulation of serine-protease Hepsin. Interestingly, Hepsin is shown to be requisite for Lkb1 loss-induced BM deterioration and branching and thus is a downstream target of Lkb1 in epithelial integrity regulation. Furthermore, the dramatic cooperation between loss of Lkb1 and c-Myc in mammary tumorigenesis appears to involve Hepsin and BM deterioration. These data suggest an interesting hypothesis that Hepsin is essential for the synergy, and aggressive tumorigenesis. Emerging data show that genes essential for the maintenance of intact epithelial integrity are often lost during human cancer progression (Reviewed by Tervonen *et al.*, 2011) and hence, it is interesting to speculate whether loss of epithelial integrity regulators leads directly to increase in cell proliferation, as has been proposed in *Drosophila* models (Bilder *et al.*, 2000), or whether they expose cells to proliferation induced by other lesions. The data presented here suggest that both types of epithelial integrity regulators exist, which may reflect their diverse functions in normal cells (IV). Also, these findings raise an interesting question of how epithelial structure regulating genes are controlling cell cycle progression.

Finally, the data presented here shows that apoptotic sensitivity is modulated by epithelial integrity (I, II), whereas the mechanisms by which c-Myc induced apoptosis in mammary epithelial cells is not. Also, in accordance with previous studies (Weaver *et al.*, 2002), the data shows that cells with disrupted epithelial integrity are more vulnerable to apoptosis. Thus, it is interesting to speculate whether this inherent sensitivity to apoptosis could be used to specifically target cancer cells. However, future studies are required to understand the underlying mechanisms of epithelial integrity in cell proliferation and apoptosis regulation.

VI ACKNOWLEDGEMENTS

This study was carried out in University of Helsinki, in the department of Biochemistry and Developmental biology at the Institute of Biomedicine in Biomedicum Helsinki and under the Genome-Scale Biology research program. I would like to thank professors Esa Korpi, Olli Jänne, Lauri Aaltonen and Tomi Mäkelä for providing the excellent facilities to carry this work through. This work has been financially supported by Graduate Program in Biotechnology and Molecular Biology (GPBM), the Academy of Finland, Sigrid Juselius foundation and TEKES as well as by Finnish Cancer Organization, Lilly Foundation, Orion-Farmos Research Foundation, K.Albin Johanssons Foundation, Waldemar von Frenckels Foundation, Paulo Foundation, Emil Aaltonen Foundation, Juliana von Wendts Foundation and Biomedicum Helsinki Foundation.

I wish to sincerely acknowledge my thesis supervisor Juha Klefström, for letting me join his team as an undergraduate student. Juha is thanked for giving me the opportunity to guide my project very independently, but also for the support and scientific guidance during these years. Importantly, Juha has encouraged me not to always follow the easiest route. Juha is also acknowledged for his endless enthusiasm towards science, including brainstorming and scientific discussions at any time of the day, which has created an inspiring atmosphere to work in.

I also would like to express my deepest gratitude to my thesis follow-up committee members Marie Henriksson and Kirsi Sainio, for their encouraging and valuable comments, which have guided me towards my thesis. Also, Marie Henriksson together with Johanna Ivaska, are warmly thanked for acting as my thesis pre-examiners and for the insightful comments and suggestions, which further strengthened my thesis. Minna Ahvenainen is warmly thanked for author-editing the English language of this thesis.

I would like to thank all my collaborators and co-authors, without whom this work would not have been possible. Tomi Mäkelä and Pekka Katajisto are thanked for their expertise and valuable insight into transgenic mice and Lkb1. Zena Werb and Peter Dijkgraaf are thanked for help with fat pad transplantation assays and mammary gland biology and Panu Kovanen for excellent help with the histopathological stainings and analyses. Outi Monni and Tanya Lepikhova are acknowledged for their excellent work with qPCR and Sampsa Hautaniemi and Marko Laakso for their invaluable input in bioinformatics.

I have had the privilege of working with very skillful technical staff: Tiina Neejärvi, Tarja Välimäki, Teija Inkinen, Leena Saikko and Ulla Kiiski are all thanked for their excellent technical expertise and help with mouse husbandry, genotyping and immunohistochemistry. Also, the past and present staff of Biomedicum Genomics and Functional Genomics unit are kindly thanked for their assistance. Additionally, secretary Anu Taulio is warmly acknowledged for her help with everyday matters as well as supplying me with all things pink.

I would especially like to thank all the members of Klefalab, with whom I have had the opportunity to work while doing my thesis. First and foremost, I would like to thank my co-authors including Anni Nieminen, Annika Hau, Topi Tervonen, Mikko Myllynen, Misa Imai, Elsa Marques and Anne Mäkelä for fruitful collaborations and scientific input. In addition, also other past and present members of Klefalab, including Vilja Eskelinen, Katriina Paasonen, Sirkku Saarikoski and semi-member Niklas Ekman are acknowledged for creating a great work atmosphere, which included passionate scientific discussions and eventful lab retreats.

Tiina Neejärvi, being always cheerful, optimistic and full with funny/crazy ideas, is especially thanked for keeping the lab running and me from not going insane. In addition to the long hours we spent together in the lab, I would also like to thank all of you for the fun times we had outside the lab as well. Truly, it has been a pleasure working with you!

Friends from neighboring Makelab and Ojala lab, including Katja, Emma, Annika, Thomas, Pekka, Kari, Anou, Lina, Tea and Kirsi are thanked for the great times we had during our joint lab retreats and other occasions. My colleagues Tea, Mira, Sanna, Can, Roxana and Anna-Maria from Biomedicum are acknowledged for great peer-support as well as for the good times we had at Faculty club, and during scientific meetings in Helsinki and abroad. Also, the former members of GSA board at GPBM are thanked for friendship and great parties. The Team Ahvis (Anni, Emma, Annika & Katja) is especially thanked for great company and the unforgettable trips to Åland, where we actually spotted some birds too.

My dearest friends Eeva, Marika, Katariina and Linnea as well as their significant others are warmly thanked for wonderful company and taking my mind off science every now and then, especially in the form of wine-tasting, girls nights out and trips abroad. Thank you for all the great times we had, and also for your encouragement (as well as putting up with me)! Also, I want to acknowledge a great group of party girls, including Sini, Paula K and Paula K, Laura H and Laura K, and Katja K for a lot of fun times. Lastly, I want to especially thank Katja Helenius, with whom I have been able to share all the disappointments and joys of science, and who has supported me through every step on my way to PhD. You are a true friend, let's never change that!

I would like to express my deepest gratitude to my parents Seija and Teemu and my stepfather Heikki, for your endless love and support in whatever I have been doing. You have inspired me so much, thank you for everything. Finally, I would like to thank Joakim for being there during my journey towards the PhD. There are not enough words to describe how grateful I am for your support. I can only say, I could not have done this without you - you are my best friend, my soul mate. I love you.

Helsinki 26.4.2012

VII REFERENCES

- Adachi, M., Inoko, A., Hata, M., Furuse, K., Umeda, K., Itoh, M., and Tsukita, S. (2006). Normal establishment of epithelial tight junctions in mice and cultured cells lacking expression of ZO-3, a tight-junction MAGUK protein. *Molecular and cellular biology* 26, 9003-9015.
- Adams, C. L., Chen, Y. T., Smith, S. J., and Nelson, W. J. (1998). Mechanisms of epithelial cell-cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin-green fluorescent protein. *The Journal of cell biology* 142, 1105-1119.
- Aijaz, S., D'Atri, F., Citi, S., Balda, M. S., and Matter, K. (2005). Binding of GEF-H1 to the tight junction-associated adaptor cingulin results in inhibition of Rho signaling and G1/S phase transition. *Developmental cell* 8, 777-786.
- Akbari, A., Razzaghi, Z., Homaei, F., Khayamzadeh, M., Movahedi, M., and Akbari, M. E. (2011). Parity and breastfeeding are preventive measures against breast cancer in Iranian women. *Breast Cancer* 18, 51-55.
- Albini, A., Johnsen, J. I., and Henriksson, M. A. (2010). MYC in oncogenesis and as a target for cancer therapies. *Advances in cancer research* 107, 163-224.
- Alcaraz, J., Mori, H., Ghajar, C. M., Brownfield, D., Galgoczy, R., and Bissell, M. J. (2011). Collective epithelial cell invasion overcomes mechanical barriers of collagenous extracellular matrix by a narrow tube-like geometry and MMP14-dependent local softening. *Integrative biology : quantitative biosciences from nano to macro* 3, 1153-1166.
- Alessi, D. R., Sakamoto, K., and Bayascas, J. R. (2006). LKB1-dependent signaling pathways. *Annual review of biochemistry* 75, 137-163.
- Alexander, A., and Walker, C. L. (2011). The role of LKB1 and AMPK in cellular responses to stress and damage. *FEBS letters* 585, 952-957.
- Amati, B., Brooks, M. W., Levy, N., Littlewood, T. D., Evan, G. I., and Land, H. (1993a). Oncogenic activity of the c-Myc protein requires dimerization with Max. *Cell* 72, 233-245.
- Amati, B., Littlewood, T. D., Evan, G. I., and Land, H. (1993b). The c-Myc protein induces cell cycle progression and apoptosis through dimerization with Max. *The EMBO journal* 12, 5083-5087.
- Amin, N., Khan, A., St Johnston, D., Tomlinson, L., Martin, S., Brenman, J., and McNeill, H. (2009). LKB1 regulates polarity remodeling and adherens junction formation in the Drosophila eye. *Proceedings of the National Academy of Sciences of the United States of America* 106, 8941-8946.
- Amundadottir, L. T., Nass, S. J., Berchem, G. J., Johnson, M. D., and Dickson, R. B. (1996). Cooperation of TGF alpha and c-Myc in mouse mammary tumorigenesis: coordinated stimulation of growth and suppression of apoptosis. *Oncogene* 13, 757-765.
- Anderson, L. H., Boulanger, C. A., Smith, G. H., Carmeliet, P., and Watson, C. J. (2011). Stem cell marker prominin-1 regulates branching morphogenesis, but not regenerative capacity, in the mammary gland. *Developmental dynamics : an official publication of the American Association of Anatomists* 240, 674-681.
- Anderson, S. M., Rudolph, M. C., McManaman, J. L., and Neville, M. C. (2007). Key stages in mammary gland development. Secretory activation in the mammary gland: it's not just about milk protein synthesis! *Breast cancer research : BCR* 9, 204.
- Andrechek, E. R., Cardiff, R. D., Chang, J. T., Gatz, M. L., Acharya, C. R., Potti, A., and Nevins, J. R. (2009). Genetic heterogeneity of Myc-induced mammary tumors reflecting diverse phenotypes including metastatic potential. *Proceedings of the National Academy of Sciences of the United States of America* 106, 16387-16392.
- Annis, M. G., Soucie, E. L., Dlugosz, P. J., Cruz-Aguado, J. A., Penn, L. Z., Leber, B., and Andrews, D. W. (2005). Bax forms multispinning monomers that oligomerize to permeabilize membranes during apoptosis. *The EMBO journal* 24, 2096-2103.
- Aretz, S., Stienen, D., Uhlhaas, S., Loff, S., Back, W., Pagenstecher, C., McLeod, D. R., Graham, G. E., Mangold, E., Santer, R., et al. (2005). High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. *Human mutation* 26, 513-519.
- Arnold, H. K., Zhang, X., Daniel, C. J., Tibbitts, D., Escamilla-Powers, J., Farrell, A., Tokarz, S., Morgan, C., and Sears, R. C. (2009). The Axin1 scaffold protein promotes formation of a degradation complex for c-Myc. *The EMBO journal* 28, 500-512.
- Arnold, I., and Watt, F. M. (2001). c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. *Current biology : CB* 11, 558-568.
- Asada, N., Sanada, K., and Fukada, Y. (2007). LKB1 regulates neuronal migration and neuronal differentiation in the developing neocortex through centrosomal positioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 27, 11769-11775.
- Askew, D. S., Ashmun, R. A., Simmons, B. C., and Cleveland, J. L. (1991). Constitutive c-myc expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. *Oncogene* 6, 1915-1922.
- Asselin-Labat, M. L., Sutherland, K. D., Barker, H., Thomas, R., Shackleton, M., Forrest, N. C., Hartley, L., Robb, L., Grosveld, F. G., van der Wees, J., et al. (2007). Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. *Nature cell biology* 9, 201-209.
- Assemet, E., Bazellieres, E., Pallesi-Pocachard, E., Le Bivic, A., and Massey-Harroche, D. (2008). Polarity complex proteins. *Biochimica et biophysica acta* 1778, 614-630.
- Aulmann, S., Adler, N., Rom, J., Helmchen, B., Schirmacher, P., and Sinn, H. P. (2006). c-myc amplifications in primary breast carcinomas and their local recurrences. *Journal of clinical pathology* 59, 424-428.
- Ayer, D. E., Kretzner, L., and Eisenman, R. N. (1993). Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity. *Cell* 72, 211-222.
- Baas, A. F., Boudeau, J., Sapkota, G. P., Smit, L., Medema, R., Morrice, N. A., Alessi, D. R., and Clevers, H. C. (2003). Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. *The EMBO journal* 22, 3062-3072.
- Baas, A. F., Kuipers, J., van der Wel, N. N., Battle, E., Koerten, H. K., Peters, P. J., and Clevers, H. C. (2004). Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell* 116, 457-466.
- Bai, L., and Rohrschneider, L. R. (2010). s-SHIP promoter expression marks activated stem cells in developing mouse mammary tissue. *Genes & development* 24, 1882-1892.
- Bar-Ner, M., Messing, L. T., Cultraro, C. M., Birrer, M. J., and Segal, S. (1992). Regions within the c-Myc protein that are necessary for transformation are also required for inhibition of differentiation of murine erythroleukemia cells. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 3, 183-190.

Baramova, E. N., Bajou, K., Remacle, A., L'Hoir, C., Krell, H. W., Weidle, U. H., Noel, A., and Foidart, J. M. (1997). Involvement of PA/plasmin system in the processing of pro-MMP-9 and in the second step of pro-MMP-2 activation. *FEBS letters* 405, 157-162.

Barcellos-Hoff, M. H., Aggeler, J., Ram, T. G., and Bissell, M. J. (1989). Functional differentiation and alveolar morphogenesis of primary mammary cultures on reconstituted basement membrane. *Development* 105, 223-235.

Bardeesy, N., Sinha, M., Hezel, A. F., Signoretti, S., Hathaway, N. A., Sharpless, N. E., Loda, M., Carrasco, D. R., and DePinho, R. A. (2002). Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. *Nature* 419, 162-167.

Barnes, A. P., Lilley, B. N., Pan, Y. A., Plummer, L. J., Powell, A. W., Raines, A. N., Sanes, J. R., and Polleux, F. (2007). LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons. *Cell* 129, 549-563.

Baudino, T. A., McKay, C., Pendeville-Samain, H., Nilsson, J. A., Maclean, K. H., White, E. L., Davis, A. C., Ihle, J. N., and Cleveland, J. L. (2002). c-Myc is essential for vasculogenesis and angiogenesis during development and tumor progression. *Genes & development* 16, 2530-2543.

Bazzoni, G., Martinez-Estrada, O. M., Orsenigo, F., Cordenonsi, M., Citi, S., and Dejana, E. (2000). Interaction of junctional adhesion molecule with the tight junction components ZO-1, cingulin, and occludin. *The Journal of biological chemistry* 275, 20520-20526.

Beer, S., Zetterberg, A., Ihrie, R. A., McTaggart, R. A., Yang, Q., Bradon, N., Arvanitis, C., Attardi, L. D., Feng, S., Ruebner, B., et al. (2004). Developmental context determines latency of MYC-induced tumorigenesis. *PLoS biology* 2, e332.

Beleut, M., Rajaram, R. D., Caikovski, M., Ayyanan, A., Germano, D., Choi, Y., Schneider, P., and Briskin, C. (2010). Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. *Proceedings of the National Academy of Sciences of the United States of America* 107, 2989-2994.

Benton, R., and St Johnston, D. (2003). A conserved oligomerization domain in drosophila Bazooka/PAR-3 is important for apical localization and epithelial polarity. *Current biology : CB* 13, 1330-1334.

Berta, M. A., Baker, C. M., Cottle, D. L., and Watt, F. M. (2010). Dose and context dependent effects of Myc on epidermal stem cell proliferation and differentiation. *EMBO molecular medicine* 2, 16-25.

Betschinger, J., Mechtler, K., and Knoblich, J. A. (2003). The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. *Nature* 422, 326-330.

Bieche, I., Laurendeau, I., Tozlu, S., Olivi, M., Vidaud, D., Lidereau, R., and Vidaud, M. (1999). Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. *Cancer research* 59, 2759-2765.

Bilder, D. (2004). Epithelial polarity and proliferation control: links from the Drosophila neoplastic tumor suppressors. *Genes & development* 18, 1909-1925.

Bilder, D., Li, M., and Perrimon, N. (2000). Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. *Science* 289, 113-116.

Bilder, D., and Perrimon, N. (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* 403, 676-680.

Blackwood, E. M., and Eisenman, R. N. (1991). Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* 251, 1211-1217.

Blakely, C. M., Sintasath, L., D'Cruz, C. M., Hahn, K. T., Dugan, K. D., Belka, G. K., and Chodosh, L. A. (2005). Developmental stage determines the effects of MYC in the mammary epithelium. *Development* 132, 1147-1160.

Blancato, J., Singh, B., Liu, A., Liao, D. J., and Dickson, R. B. (2004). Correlation of amplification and overexpression of the c-myc oncogene in high-grade breast cancer: FISH, in situ hybridisation and immunohistochemical analyses. *British journal of cancer* 90, 1612-1619.

Bole-Feysot, C., Goffin, V., Edery, M., Binart, N., and Kelly, P. A. (1998). Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocrine reviews* 19, 225-268.

Boras-Granic, K., Chang, H., Grosschedl, R., and Hamel, P. A. (2006). Lef1 is required for the transition of Wnt signaling from mesenchymal to epithelial cells in the mouse embryonic mammary gland. *Developmental biology* 295, 219-231.

Boudeau, J., Baas, A. F., Deak, M., Morrice, N. A., Kieloch, A., Schutkowski, M., Prescott, A. R., Clevers, H. C., and Alessi, D. R. (2003). MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm. *The EMBO journal* 22, 5102-5114.

Boudreau, N., Werb, Z., and Bissell, M. J. (1996). Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. *Proceedings of the National Academy of Sciences of the United States of America* 93, 3509-3513.

Boyd, N. F., Martin, L. J., Yaffe, M. J., and Minkin, S. (2011). Mammographic density and breast cancer risk: current understanding and future prospects. *Breast cancer research : BCR* 13, 223.

Braga, V. M., Machesky, L. M., Hall, A., and Hotchin, N. A. (1997). The small GTPases Rho and Rac are required for the establishment of cadherin-dependent cell-cell contacts. *The Journal of cell biology* 137, 1421-1431.

Briskin, C., Heineman, A., Chavarria, T., Elenbaas, B., Tan, J., Dey, S. K., McMahon, J. A., McMahon, A. P., and Weinberg, R. A. (2000). Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. *Genes & development* 14, 650-654.

Briskin, C., and O'Malley, B. (2010). Hormone action in the mammary gland. *Cold Spring Harbor perspectives in biology* 2, a003178.

Briskin, C., Park, S., Vass, T., Lydon, J. P., O'Malley, B. W., and Weinberg, R. A. (1998). A paracrine role for the epithelial progesterone receptor in mammary gland development. *Proceedings of the National Academy of Sciences of the United States of America* 95, 5076-5081.

Brody, T. (1999). The Interactive Fly: gene networks, development and the Internet. *Trends in genetics : TIG* 15, 333-334.

Brumby, A. M., and Richardson, H. E. (2003). scribble mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in Drosophila. *The EMBO journal* 22, 5769-5779.

Brumby, A. M., and Richardson, H. E. (2005). Using Drosophila melanogaster to map human cancer pathways. *Nature reviews Cancer* 5, 626-639.

Brunner, T., Kasibhatla, S., Pinkoski, M. J., Fruttschi, C., Yoo, N. J., Echeverri, F., Mahboubi, A., and Green, D. R. (2000). Expression of Fas ligand in activated T cells is regulated by c-Myc. *The Journal of biological chemistry* 275, 9767-9772.

Bryant, D. M., Datta, A., Rodriguez-Fraticelli, A. E., Peranen, J., Martin-Belmonte, F., and Mostov, K. E. (2010). A molecular network for de novo generation of the apical surface and lumen. *Nature cell biology* *12*, 1035-1045.

Bryant, D. M., and Mostov, K. E. (2008). From cells to organs: building polarized tissue. *Nature reviews Molecular cell biology* *9*, 887-901.

Bui, T. V., and Mendell, J. T. (2010). Myc: Maestro of MicroRNAs. *Genes & cancer* *1*, 568-575.

Butt, S., Borgquist, S., Anagnostaki, L., Landberg, G., and Manjer, J. (2009). Parity and age at first childbirth in relation to the risk of different breast cancer subgroups. *International journal of cancer Journal international du cancer* *125*, 1926-1934.

Campbell, S. M., Rosen, J. M., Hennighausen, L. G., Strech-Jurk, U., and Sippel, A. E. (1984). Comparison of the whey acidic protein genes of the rat and mouse. *Nucleic acids research* *12*, 8685-8697.

Cavey, M., and Lecuit, T. (2009). Molecular bases of cell-cell junctions stability and dynamics. *Cold Spring Harbor perspectives in biology* *1*, a002998.

Chen, C. L., Gajewski, K. M., Hamaratoglu, F., Bossuyt, W., Sansores-Garcia, L., Tao, C., and Halder, G. (2010). The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America* *107*, 15810-15815.

Chen, J., Diacovo, T. G., Grenache, D. G., Santoro, S. A., and Zutter, M. M. (2002). The alpha(2) integrin subunit-deficient mouse: a multifaceted phenotype including defects of branching morphogenesis and hemostasis. *The American journal of pathology* *161*, 337-344.

Chen, L. H., and Bissell, M. J. (1989). A novel regulatory mechanism for whey acidic protein gene expression. *Cell regulation* *1*, 45-54.

Chen, X., and Macara, I. G. (2005). Par-3 controls tight junction assembly through the Rac exchange factor Tiam1. *Nature cell biology* *7*, 262-269.

Cheng, H., Liu, P., Wang, Z. C., Zou, L., Santiago, S., Garbitt, V., Gjoerup, O. V., Iglehart, J. D., Miron, A., Richardson, A. L., *et al.* (2009). SIK1 couples LKB1 to p53-dependent anoikis and suppresses metastasis. *Science signaling* *2*, ra35.

Cheng, S. W., Davies, K. P., Yung, E., Beltran, R. J., Yu, J., and Kalpana, G. V. (1999). c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. *Nature genetics* *22*, 102-105.

Chitaev, N. A., and Troyanovsky, S. M. (1997). Direct Ca²⁺-dependent heterophilic interaction between desmosomal cadherins, desmoglein and desmocollin, contributes to cell-cell adhesion. *The Journal of cell biology* *138*, 193-201.

Chrzan, P., Skokowski, J., Karmolinski, A., and Pawelczyk, T. (2001). Amplification of c-myc gene and overexpression of c-Myc protein in breast cancer and adjacent non-neoplastic tissue. *Clinical biochemistry* *34*, 557-562.

Chu, E. Y., Hens, J., Andl, T., Kairo, A., Yamaguchi, T. P., Briskin, C., Glick, A., Wysolmerski, J. J., and Millar, S. E. (2004). Canonical WNT signaling promotes mammary placode development and is essential for initiation of mammary gland morphogenesis. *Development* *131*, 4819-4829.

Ciarloni, L., Mallepell, S., and Briskin, C. (2007). Amphiregulin is an essential mediator of estrogen receptor alpha function in mammary gland development. *Proceedings of the National Academy of Sciences of the United States of America* *104*, 5455-5460.

Conde, E., Suarez-Gauthier, A., Garcia-Garcia, E., Lopez-Rios, F., Lopez-Encuentra, A., Garcia-Lujan, R., Morente, M., Sanchez-Verde, L., and Sanchez-Cespedes, M. (2007). Specific pattern of LKB1 and phospho-acetyl-CoA carboxylase protein immunostaining in human normal tissues and lung carcinomas. *Human pathology* *38*, 1351-1360.

Contreras, C. M., Akbay, E. A., Gallardo, T. D., Haynie, J. M., Sharma, S., Tagao, O., Bardeesy, N., Takahashi, M., Settleman, J., Wong, K. K., and Castrillon, D. H. (2010). Lkb1 inactivation is sufficient to drive endometrial cancers that are aggressive yet highly responsive to mTOR inhibitor monotherapy. *Disease models & mechanisms* *3*, 181-193.

Contreras, C. M., Gurumurthy, S., Haynie, J. M., Shirley, L. J., Akbay, E. A., Wingo, S. N., Schorge, J. O., Broaddus, R. R., Wong, K. K., Bardeesy, N., and Castrillon, D. H. (2008). Loss of Lkb1 provokes highly invasive endometrial adenocarcinomas. *Cancer research* *68*, 759-766.

Corzo, C., Corominas, J. M., Tusquets, I., Salido, M., Bellet, M., Fabregat, X., Serrano, S., and Sole, F. (2006). The MYC oncogene in breast cancer progression: from benign epithelium to invasive carcinoma. *Cancer genetics and cytogenetics* *165*, 151-156.

Cowling, V. H., and Cole, M. D. (2007). The Myc transactivation domain promotes global phosphorylation of the RNA polymerase II carboxy-terminal domain independently of direct DNA binding. *Molecular and cellular biology* *27*, 2059-2073.

D'Cruz, C. M., Gunther, E. J., Boxer, R. B., Hartman, J. L., Sintasath, L., Moody, S. E., Cox, J. D., Ha, S. I., Belka, G. K., Golant, A., *et al.* (2001). c-MYC induces mammary tumorigenesis by means of a preferred pathway involving spontaneous Kras2 mutations. *Nature medicine* *7*, 235-239.

Daley, W. P., Gervais, E. M., Centanni, S. W., Gulfo, K. M., Nelson, D. A., and Larsen, M. (2012). ROCK1-directed basement membrane positioning coordinates epithelial tissue polarity. *Development* *139*, 411-422.

Dalglish, G. L., Furge, K., Greenman, C., Chen, L., Bignell, G., Butler, A., Davies, H., Edkins, S., Hardy, C., Latimer, C., *et al.* (2010). Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. *Nature* *463*, 360-363.

Dang, C. V. (2012). MYC on the Path to Cancer. *Cell* *149*, 22-35.

Dang, C. V., O'Donnell, K. A., Zeller, K. I., Nguyen, T., Osthus, R. C., and Li, F. (2006). The c-Myc target gene network. *Seminars in cancer biology* *16*, 253-264.

Dani, C., Blanchard, J. M., Piechaczyk, M., El Sabouty, S., Marty, L., and Jeanteur, P. (1984). Extreme instability of myc mRNA in normal and transformed human cells. *Proceedings of the National Academy of Sciences of the United States of America* *81*, 7046-7050.

Daniel, C. W., Silberstein, G. B., and Strickland, P. (1987). Direct action of 17 beta-estradiol on mouse mammary ducts analyzed by sustained release implants and steroid autoradiography. *Cancer research* *47*, 6052-6057.

Daniel, C. W., Silberstein, G. B., Van Horn, K., Strickland, P., and Robinson, S. (1989). TGF-beta 1-induced inhibition of mouse mammary ductal growth: developmental specificity and characterization. *Developmental biology* *135*, 20-30.

Dansen, T. B., Whitfield, J., Rostker, F., Brown-Swigart, L., and Evan, G. I. (2006). Specific requirement for Bax, not Bak, in Myc-induced apoptosis and tumor suppression in vivo. *The Journal of biological chemistry* *281*, 10890-10895.

Davis, L. J., and Halazonetis, T. D. (1993). Both the helix-loop-helix and the leucine zipper motifs of c-Myc contribute to its dimerization specificity with Max. *Oncogene* *8*, 125-132.

Dean, M., Levine, R. A., Ran, W., Kindy, M. S., Sonenshein, G. E., and Campisi, J. (1986). Regulation of c-myc transcription and mRNA abundance by serum growth factors and cell contact. *The Journal of biological chemistry* 261, 9161-9166.

Debnath, J., Mills, K. R., Collins, N. L., Reginato, M. J., Muthuswamy, S. K., and Brugge, J. S. (2002). The role of apoptosis in creating and maintaining luminal space within normal and oncogene-expressing mammary acini. *Cell* 111, 29-40.

Debnath, J., Muthuswamy, S. K., and Brugge, J. S. (2003a). Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods* 30, 256-268.

Debnath, J., Walker, S. J., and Brugge, J. S. (2003b). Akt activation disrupts mammary acinar architecture and enhances proliferation in an mTOR-dependent manner. *The Journal of cell biology* 163, 315-326.

Delehedde, M., Lyon, M., Sergeant, N., Rahmoune, H., and Fernig, D. G. (2001). Proteoglycans: pericellular and cell surface multireceptors that integrate external stimuli in the mammary gland. *Journal of mammary gland biology and neoplasia* 6, 253-273.

Delgado, M. D., Lerga, A., Canelles, M., Gomez-Casares, M. T., and Leon, J. (1995). Differential regulation of Max and role of c-Myc during erythroid and myelomonocytic differentiation of K562 cells. *Oncogene* 10, 1659-1665.

den Elzen, N., BATTERY, C. V., Maddugoda, M. P., Ren, G., and Yap, A. S. (2009). Cadherin adhesion receptors orient the mitotic spindle during symmetric cell division in mammalian epithelia. *Molecular biology of the cell* 20, 3740-3750.

Denef, N., Chen, Y., Weeks, S. D., Barcelo, G., and Schupbach, T. (2008). Crag regulates epithelial architecture and polarized deposition of basement membrane proteins in *Drosophila*. *Developmental cell* 14, 354-364.

Deome, K. B., Faulkin, L. J., Jr., Bern, H. A., and Blair, P. B. (1959). Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. *Cancer research* 19, 515-520.

Dhanasekaran, S. M., Barrette, T. R., Ghosh, D., Shah, R., Varambally, S., Kurachi, K., Pienta, K. J., Rubin, M. A., and Chinnaiyan, A. M. (2001). Delineation of prognostic biomarkers in prostate cancer. *Nature* 412, 822-826.

Dmitrovsky, E., Kuehl, W. M., Hollis, G. F., Kirsch, I. R., Bender, T. P., and Segal, S. (1986). Expression of a transfected human c-myc oncogene inhibits differentiation of a mouse erythroleukaemia cell line. *Nature* 322, 748-750.

Dolberg, D. S., and Bissell, M. J. (1984). Inability of Rous sarcoma virus to cause sarcomas in the avian embryo. *Nature* 309, 552-556.

Dong, J., Tong, T., Reynado, A. M., Rosen, J. M., Huang, S., and Li, Y. (2010). Genetic manipulation of individual somatic mammary cells in vivo reveals a master role of STAT5a in inducing alveolar fate commitment and lactogenesis even in the absence of ovarian hormones. *Developmental biology* 346, 196-203.

Dorfman, J., and Macara, I. G. (2008). STRADalpha regulates LKB1 localization by blocking access to importin-alpha, and by association with Crm1 and exportin-7. *Molecular biology of the cell* 19, 1614-1626.

Drees, F., Pokutta, S., Yamada, S., Nelson, W. J., and Weis, W. I. (2005). Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. *Cell* 123, 903-915.

Du, J. Y., Chen, M. C., Hsu, T. C., Wang, J. H., Brackenbury, L., Lin, T. H., Wu, Y. Y., Yang, Z., Streuli, C. H., and Lee, Y. J. (2012). The RhoA-Rok-myosin II pathway is involved in extracellular matrix-mediated regulation of prolactin signaling in mammary epithelial cells. *Journal of cellular physiology* 227, 1553-1560.

Dunbar, M. E., and Wysolmerski, J. J. (1999). Parathyroid hormone-related protein: a developmental regulatory molecule necessary for mammary gland development. *Journal of mammary gland biology and neoplasia* 4, 21-34.

Durgan, J., Kaji, N., Jin, D., and Hall, A. (2011). Par6B and atypical PKC regulate mitotic spindle orientation during epithelial morphogenesis. *The Journal of biological chemistry* 286, 12461-12474.

Easton, D. F., Pooley, K. A., Dunning, A. M., Pharoah, P. D., Thompson, D., Ballinger, D. G., Struwing, J. P., Morrison, J., Field, H., Luben, R., *et al.* (2007). Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature* 447, 1087-1093.

Eberhardy, S. R., and Farnham, P. J. (2002). Myc recruits P-TEFb to mediate the final step in the transcriptional activation of the cad promoter. *The Journal of biological chemistry* 277, 40156-40162.

Eder, A. M., Sui, X., Rosen, D. G., Nolden, L. K., Cheng, K. W., Lahad, J. P., Kango-Singh, M., Lu, K. H., Warneke, C. L., Atkinson, E. N., *et al.* (2005). Atypical PKC α contributes to poor prognosis through loss of apical-basal polarity and cyclin E overexpression in ovarian cancer. *Proceedings of the National Academy of Sciences of the United States of America* 102, 12519-12524.

Edwards, G. M., Wilford, F. H., Liu, X., Hennighausen, L., Djiane, J., and Streuli, C. H. (1998). Regulation of mammary differentiation by extracellular matrix involves protein-tyrosine phosphatases. *The Journal of biological chemistry* 273, 9495-9500.

Edwards, G. M., Wilford, F. H., and Streuli, C. H. (1996). Extracellular matrix controls the prolactin signalling pathway in mammary epithelial cells. *Biochemical Society transactions* 24, 345S.

Egle, A., Harris, A. W., Bouillet, P., and Cory, S. (2004). Bim is a suppressor of Myc-induced mouse B cell leukemia. *Proceedings of the National Academy of Sciences of the United States of America* 101, 6164-6169.

Eilers, M., and Eisenman, R. N. (2008). Myc's broad reach. *Genes & development* 22, 2755-2766.

Eilers, M., Picard, D., Yamamoto, K. R., and Bishop, J. M. (1989). Chimaeras of myc oncoprotein and steroid receptors cause hormone-dependent transformation of cells. *Nature* 340, 66-68.

Eischen, C. M., Packham, G., Nip, J., Fee, B. E., Hiebert, S. W., Zambetti, G. P., and Cleveland, J. L. (2001a). Bcl-2 is an apoptotic target suppressed by both c-Myc and E2F-1. *Oncogene* 20, 6983-6993.

Eischen, C. M., Roussel, M. F., Korsmeyer, S. J., and Cleveland, J. L. (2001b). Bax loss impairs Myc-induced apoptosis and circumvents the selection of p53 mutations during Myc-mediated lymphomagenesis. *Molecular and cellular biology* 21, 7653-7662.

El-Sabban, M. E., Sfeir, A. J., Daher, M. H., Kalaany, N. Y., Bassam, R. A., and Talhouk, R. S. (2003). ECM-induced gap junctional communication enhances mammary epithelial cell differentiation. *Journal of cell science* 116, 3531-3541.

Esteller, M., Avizienyte, E., Corn, P. G., Lothe, R. A., Baylin, S. B., Aaltonen, L. A., and Herman, J. G. (2000). Epigenetic inactivation of LKB1 in primary tumors associated with the Peutz-Jeghers syndrome. *Oncogene* 19, 164-168.

Etienne-Manneville, S. (2008). Polarity proteins in migration and invasion. *Oncogene* 27, 6970-6980.

Evan, G. I., Wyllie, A. H., Gilbert, C. S., Littlewood, T. D., Land, H., Brooks, M., Waters, C. M., Penn, L. Z., and Hancock, D. C. (1992). Induction of apoptosis in fibroblasts by c-myc protein. *Cell* 69, 119-128.

- Evans, J. D., Cornford, P. A., Dodson, A., Neoptolemos, J. P., and Foster, C. S. (2003). Expression patterns of protein kinase C isoenzymes are characteristically modulated in chronic pancreatitis and pancreatic cancer. *American journal of clinical pathology* 119, 392-402.
- Ewald, A. J., Brenot, A., Duong, M., Chan, B. S., and Werb, Z. (2008). Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. *Developmental cell* 14, 570-581.
- Fang, C. M., and Xu, Y. H. (2001). Down-regulated expression of atypical PKC-binding domain deleted asip isoforms in human hepatocellular carcinomas. *Cell research* 11, 223-229.
- Fanning, A. S., Jameson, B. J., Jesaitis, L. A., and Anderson, J. M. (1998). The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. *The Journal of biological chemistry* 273, 29745-29753.
- Fanning, A. S., Ma, T. Y., and Anderson, J. M. (2002). Isolation and functional characterization of the actin binding region in the tight junction protein ZO-1. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 16, 1835-1837.
- Fata, J. E., Leco, K. J., Moorehead, R. A., Martin, D. C., and Khokha, R. (1999). Timp-1 is important for epithelial proliferation and branching morphogenesis during mouse mammary development. *Developmental biology* 211, 238-254.
- Fata, J. E., Werb, Z., and Bissell, M. J. (2004). Regulation of mammary gland branching morphogenesis by the extracellular matrix and its remodeling enzymes. *Breast cancer research : BCR* 6, 1-11.
- Feldman, M., Ruan, W., Tappin, I., Wiczorek, R., and Kleinberg, D. L. (1999). The effect of GH on estrogen receptor expression in the rat mammary gland. *The Journal of endocrinology* 163, 515-522.
- Felsher, D. W., and Bishop, J. M. (1999). Transient excess of MYC activity can elicit genomic instability and tumorigenesis. *Proceedings of the National Academy of Sciences of the United States of America* 96, 3940-3944.
- Feng, Y., Manka, D., Wagner, K. U., and Khan, S. A. (2007). Estrogen receptor-alpha expression in the mammary epithelium is required for ductal and alveolar morphogenesis in mice. *Proceedings of the National Academy of Sciences of the United States of America* 104, 14718-14723.
- Fenton, H., Carlile, B., Montgomery, E. A., Carraway, H., Herman, J., Sahin, F., Su, G. H., and Argani, P. (2006). LKB1 protein expression in human breast cancer. *Applied immunohistochemistry & molecular morphology : AIMM / official publication of the Society for Applied Immunohistochemistry* 14, 146-153.
- Fernandez, P. C., Frank, S. R., Wang, L., Schroeder, M., Liu, S., Greene, J., Cocito, A., and Amati, B. (2003). Genomic targets of the human c-Myc protein. *Genes & development* 17, 1115-1129.
- Fogg, V. C., Liu, C. J., and Margolis, B. (2005). Multiple regions of Crumbs3 are required for tight junction formation in MCF10A cells. *Journal of cell science* 118, 2859-2869.
- Foley, J., Dann, P., Hong, J., Cosgrove, J., Dreyer, B., Rimm, D., Dunbar, M., Philbrick, W., and Wysolmerski, J. (2001). Parathyroid hormone-related protein maintains mammary epithelial fate and triggers nipple skin differentiation during embryonic breast development. *Development* 128, 513-525.
- Forster, L. F., Defres, S., Goudie, D. R., Baty, D. U., and Carey, F. A. (2000). An investigation of the Peutz-Jeghers gene (LKB1) in sporadic breast and colon cancers. *Journal of clinical pathology* 53, 791-793.
- Freytag, S. O., Dang, C. V., and Lee, W. M. (1990). Definition of the activities and properties of c-myc required to inhibit cell differentiation. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 1, 339-343.
- Fuja, T. J., Lin, F., Osann, K. E., and Bryant, P. J. (2004). Somatic mutations and altered expression of the candidate tumor suppressors CSNK1 epsilon, DLG1, and EDD/hYD in mammary ductal carcinoma. *Cancer research* 64, 942-951.
- Futreal, P. A., Liu, Q., Shattuck-Eidens, D., Cochran, C., Harshman, K., Tavtigian, S., Bennett, L. M., Haugen-Strano, A., Swensen, J., Miki, Y., and et al. (1994). BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 266, 120-122.
- Gallego, M. I., Binart, N., Robinson, G. W., Okagaki, R., Coschigano, K. T., Perry, J., Kopchick, J. J., Oka, T., Kelly, P. A., and Hennighausen, L. (2001). Prolactin, growth hormone, and epidermal growth factor activate Stat5 in different compartments of mammary tissue and exert different and overlapping developmental effects. *Developmental biology* 229, 163-175.
- Gangar, A., Rossi, G., Andreeva, A., Hales, R., and Brennwald, P. (2005). Structurally conserved interaction of Lgl family with SNAREs is critical to their cellular function. *Current biology : CB* 15, 1136-1142.
- Gao, B., Sun, Y., Zhang, J., Ren, Y., Fang, R., Han, X., Shen, L., Liu, X. Y., Pao, W., Chen, H., and Ji, H. (2010a). Spectrum of LKB1, EGFR, and KRAS mutations in chinese lung adenocarcinomas. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 5, 1130-1135.
- Gao, L., Joberty, G., and Macara, I. G. (2002). Assembly of epithelial tight junctions is negatively regulated by Par6. *Current biology : CB* 12, 221-225.
- Gao, L., and Macara, I. G. (2004). Isoforms of the polarity protein par6 have distinct functions. *The Journal of biological chemistry* 279, 41557-41562.
- Gao, Y., Xiao, Q., Ma, H., Li, L., Liu, J., Feng, Y., Fang, Z., Wu, J., Han, X., Zhang, J., et al. (2010b). LKB1 inhibits lung cancer progression through lysyl oxidase and extracellular matrix remodeling. *Proceedings of the National Academy of Sciences of the United States of America* 107, 18892-18897.
- Gardioli, D., Kuhne, C., Glaunsinger, B., Lee, S. S., Javier, R., and Banks, L. (1999). Oncogenic human papillomavirus E6 proteins target the discs large tumour suppressor for proteasome-mediated degradation. *Oncogene* 18, 5487-5496.
- Gardioli, D., Zacchi, A., Petrera, F., Stanta, G., and Banks, L. (2006). Human discs large and scrib are localized at the same regions in colon mucosa and changes in their expression patterns are correlated with loss of tissue architecture during malignant progression. *International journal of cancer Journal international du cancer* 119, 1285-1290.
- Garner, O. B., Bush, K. T., Nigam, K. B., Yamaguchi, Y., Xu, D., Esko, J. D., and Nigam, S. K. (2011). Stage-dependent regulation of mammary ductal branching by heparan sulfate and HGF-cMet signaling. *Developmental biology* 355, 394-403.
- Garrod, D., and Chidgey, M. (2008). Desmosome structure, composition and function. *Biochimica et biophysica acta* 1778, 572-587.
- Gasco, M., Shami, S., and Crook, T. (2002). The p53 pathway in breast cancer. *Breast cancer research : BCR* 4, 70-76.
- Gassama-Diagne, A., Yu, W., ter Beest, M., Martin-Belmonte, F., Kierbel, A., Engel, J., and Mostov, K. (2006). Phosphatidylinositol-3,4,5-trisphosphate regulates the formation of the basolateral plasma membrane in epithelial cells. *Nature cell biology* 8, 963-970.

Gateff, E. (1994). Tumor suppressor and overgrowth suppressor genes of *Drosophila melanogaster*: developmental aspects. *The International journal of developmental biology* 38, 565-590.

Giardiello, F. M., Welsh, S. B., Hamilton, S. R., Offerhaus, G. J., Gittelsohn, A. M., Booker, S. V., Krush, A. J., Yardley, J. H., and Luk, G. D. (1987). Increased risk of cancer in the Peutz-Jeghers syndrome. *The New England journal of medicine* 316, 1511-1514.

Goldstein, B., and Macara, I. G. (2007). The PAR proteins: fundamental players in animal cell polarization. *Developmental cell* 13, 609-622.

Gomez-Roman, N., Grandori, C., Eisenman, R. N., and White, R. J. (2003). Direct activation of RNA polymerase III transcription by c-Myc. *Nature* 421, 290-294.

Gonzalez-Mariscal, L., Betanzos, A., Nava, P., and Jaramillo, B. E. (2003). Tight junction proteins. *Progress in biophysics and molecular biology* 81, 1-44.

Gopalakrishnan, S., Raman, N., Atkinson, S. J., and Marrs, J. A. (1998). Rho GTPase signaling regulates tight junction assembly and protects tight junctions during ATP depletion. *The American journal of physiology* 275, C798-809.

Granot, Z., Swisa, A., Magenheimer, J., Stolovich-Rain, M., Fujimoto, W., Manduchi, E., Miki, T., Lennerz, J. K., Stoeckert, C. J., Jr., Meyuhas, O., *et al.* (2009). LKB1 regulates pancreatic beta cell size, polarity, and function. *Cell metabolism* 10, 296-308.

Grassian, A. R., Metallo, C. M., Coloff, J. L., Stephanopoulos, G., and Brugge, J. S. (2011). Erk regulation of pyruvate dehydrogenase flux through PDK4 modulates cell proliferation. *Genes & development* 25, 1716-1733.

Gray, R. S., Cheung, K. J., and Ewald, A. J. (2010). Cellular mechanisms regulating epithelial morphogenesis and cancer invasion. *Current opinion in cell biology* 22, 640-650.

Greer, E. L., Oskoui, P. R., Banko, M. R., Maniar, J. M., Gygi, M. P., Gygi, S. P., and Brunet, A. (2007). The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. *The Journal of biological chemistry* 282, 30107-30119.

Gregory, M. A., Qi, Y., and Hann, S. R. (2003). Phosphorylation by glycogen synthase kinase-3 controls c-myc proteolysis and subnuclear localization. *The Journal of biological chemistry* 278, 51606-51612.

Grifoni, D., Garoia, F., Bellosta, P., Parisi, F., De Biase, D., Collina, G., Strand, D., Cavicchi, S., and Pession, A. (2007). aPKCzeta cortical loading is associated with Lgl cytoplasmic release and tumor growth in *Drosophila* and human epithelia. *Oncogene* 26, 5960-5965.

Grushko, T. A., Dignam, J. J., Das, S., Blackwood, A. M., Perou, C. M., Ridderstrale, K. K., Anderson, K. N., Wei, M. J., Adams, A. J., Hagos, F. G., *et al.* (2004). MYC is amplified in BRCA1-associated breast cancers. *Clinical cancer research : an official journal of the American Association for Cancer Research* 10, 499-507.

Gulberg, P., Thor Straten, P., Ahrenkiel, V., Seremet, T., Kirkin, A. F., and Zeuthen, J. (1999). Somatic mutation of the Peutz-Jeghers syndrome gene, LKB1/STK11, in malignant melanoma. *Oncogene* 18, 1777-1780.

Guo, S., and Kemphues, K. J. (1995). par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* 81, 611-620.

Gurumurthy, S., Hezel, A. F., Sahin, E., Berger, J. H., Bosenberg, M. W., and Bardeesy, N. (2008). LKB1 deficiency sensitizes mice to carcinogen-induced tumorigenesis. *Cancer research* 68, 55-63.

Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., Turk, B. E., and Shaw, R. J. (2008). AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Molecular cell* 30, 214-226.

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646-674.

Hann, S. R., and Eisenman, R. N. (1984). Proteins encoded by the human c-myc oncogene: differential expression in neoplastic cells. *Molecular and cellular biology* 4, 2486-2497.

Hao, Y., Du, Q., Chen, X., Zheng, Z., Balsbaugh, J. L., Maitra, S., Shabanowitz, J., Hunt, D. F., and Macara, I. G. (2010). Par3 controls epithelial spindle orientation by aPKC-mediated phosphorylation of apical Pins. *Current biology : CB* 20, 1809-1818.

Hariharan, I. K., and Bilder, D. (2006). Regulation of imaginal disc growth by tumor-suppressor genes in *Drosophila*. *Annual review of genetics* 40, 335-361.

Harris, J., Stanford, P. M., Sutherland, K., Oakes, S. R., Naylor, M. J., Robertson, F. G., Blazek, K. D., Kazlauskas, M., Hilton, H. N., Wittlin, S., *et al.* (2006). Socs2 and elf5 mediate prolactin-induced mammary gland development. *Mol Endocrinol* 20, 1177-1187.

Harvey, K. F., Pfeleger, C. M., and Hariharan, I. K. (2003). The *Drosophila* Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis. *Cell* 114, 457-467.

Haslam, S. Z. (1989). The ontogeny of mouse mammary gland responsiveness to ovarian steroid hormones. *Endocrinology* 125, 2766-2772.

Haslam, S. Z., and Nummy, K. A. (1992). The ontogeny and cellular distribution of estrogen receptors in normal mouse mammary gland. *The Journal of steroid biochemistry and molecular biology* 42, 589-595.

Hawley, S. A., Boudeau, J., Reid, J. L., Mustard, K. J., Udd, L., Makela, T. P., Alessi, D. R., and Hardie, D. G. (2003). Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *Journal of biology* 2, 28.

Hearle, N., Schumacher, V., Menko, F. H., Olschwang, S., Boardman, L. A., Gille, J. J., Keller, J. J., Westerman, A. M., Scott, R. J., Lim, W., *et al.* (2006). Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. *Clinical cancer research : an official journal of the American Association for Cancer Research* 12, 3209-3215.

Hemann, M. T., Bric, A., Teruya-Feldstein, J., Herbst, A., Nilsson, J. A., Cordon-Cardo, C., Cleveland, J. L., Tansey, W. P., and Lowe, S. W. (2005). Evasion of the p53 tumour surveillance network by tumour-derived MYC mutants. *Nature* 436, 807-811.

Hemminki, A., Markie, D., Tomlinson, I., Avizienyte, E., Roth, S., Loukola, A., Bignell, G., Warren, W., Aminoff, M., Hoglund, P., *et al.* (1998). A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature* 391, 184-187.

Hennighausen, L., and Robinson, G. W. (2001). Signaling pathways in mammary gland development. *Developmental cell* 1, 467-475.

Henriksson, M., Bakardjiev, A., Klein, G., and Luscher, B. (1993). Phosphorylation sites mapping in the N-terminal domain of c-myc modulate its transforming potential. *Oncogene* 8, 3199-3209.

Hens, J. R., and Wysolmerski, J. J. (2005). Key stages of mammary gland development: molecular mechanisms involved in the formation of the embryonic mammary gland. *Breast cancer research : BCR* 7, 220-224.

Hermeking, H., and Eick, D. (1994). Mediation of c-Myc-induced apoptosis by p53. *Science* 265, 2091-2093.

Herschkowitz, J. I., Simin, K., Weigman, V. J., Mikaelian, I., Usary, J., Hu, Z., Rasmussen, K. E., Jones, L. P., Assefnia, S., Chandrasekharan, S., *et al.* (2007). Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. *Genome biology* 8, R76.

Herve, J. C., Bourmeyster, N., Sarrouilhe, D., and Duffy, H. S. (2007). Gap junctional complexes: from partners to functions. *Progress in biophysics and molecular biology* 94, 29-65.

Hezel, A. F., Gurumurthy, S., Granot, Z., Swisa, A., Chu, G. C., Bailey, G., Dor, Y., Bardeesy, N., and Depinho, R. A. (2008). Pancreatic LKB1 deletion leads to acinar polarity defects and cystic neoplasms. *Molecular and cellular biology* 28, 2414-2425.

Hinck, L., and Silberstein, G. B. (2005). Key stages in mammary gland development: the mammary end bud as a motile organ. *Breast cancer research : BCR* 7, 245-251.

Hirose, T., Izumi, Y., Nagashima, Y., Tamai-Nagai, Y., Kurihara, H., Sakai, T., Suzuki, Y., Yamanaka, T., Suzuki, A., Mizuno, K., and Ohno, S. (2002). Involvement of ASIP/Par-3 in the promotion of epithelial tight junction formation. *Journal of cell science* 115, 2485-2495.

Hoffman, B., and Liebermann, D. A. (2008). Apoptotic signaling by c-MYC. *Oncogene* 27, 6462-6472.

Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. (1991). p53 mutations in human cancers. *Science* 253, 49-53.

Hopkinson, S. B., and Jones, J. C. (2000). The N terminus of the transmembrane protein BP180 interacts with the N-terminal domain of BP230, thereby mediating keratin cytoskeleton anchorage to the cell surface at the site of the hemidesmosome. *Molecular biology of the cell* 11, 277-286.

Horikoshi, Y., Suzuki, A., Yamanaka, T., Sasaki, K., Mizuno, K., Sawada, H., Yonemura, S., and Ohno, S. (2009). Interaction between PAR-3 and the aPKC-PAR-6 complex is indispensable for apical domain development of epithelial cells. *Journal of cell science* 122, 1595-1606.

Hotary, K., Li, X. Y., Allen, E., Stevens, S. L., and Weiss, S. J. (2006). A cancer cell metalloprotease triad regulates the basement membrane transmigration program. *Genes & development* 20, 2673-2686.

Hovey, R. C., Trott, J. F., Ginsburg, E., Goldhar, A., Sasaki, M. M., Fountain, S. J., Sundararajan, K., and Vonderhaar, B. K. (2001). Transcriptional and spatiotemporal regulation of prolactin receptor mRNA and cooperativity with progesterone receptor function during ductal branch growth in the mammary gland. *Developmental dynamics : an official publication of the American Association of Anatomists* 222, 192-205.

Howlett, A. R., and Bissell, M. J. (1993). The influence of tissue microenvironment (stroma and extracellular matrix) on the development and function of mammary epithelium. *Epithelial cell biology* 2, 79-89.

Hsu, B., Marin, M. C., el-Naggar, A. K., Stephens, L. C., Brisbay, S., and McDonnell, T. J. (1995). Evidence that c-myc mediated apoptosis does not require wild-type p53 during lymphomagenesis. *Oncogene* 11, 175-179.

Huang, H., Potter, C. J., Tao, W., Li, D. M., Brogiolo, W., Hafen, E., Sun, H., and Xu, T. (1999). PTEN affects cell size, cell proliferation and apoptosis during Drosophila eye development. *Development* 126, 5365-5372.

Huang, X., Wullschlegel, S., Shpiro, N., McGuire, V. A., Sakamoto, K., Woods, Y. L., McBurnie, W., Fleming, S., and Alessi, D. R. (2008). Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice. *The Biochemical journal* 412, 211-221.

Hueber, A. O., Zornig, M., Lyon, D., Suda, T., Nagata, S., and Evan, G. I. (1997). Requirement for the CD95 receptor-ligand pathway in c-Myc-induced apoptosis. *Science* 278, 1305-1309.

Hung, T. J., and Kemphues, K. J. (1999). PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in *Caenorhabditis elegans* embryos. *Development* 126, 127-135.

Hurd, T. W., Gao, L., Roh, M. H., Macara, I. G., and Margolis, B. (2003). Direct interaction of two polarity complexes implicated in epithelial tight junction assembly. *Nature cell biology* 5, 137-142.

Hurov, J. B., Watkins, J. L., and Piwnicka-Worms, H. (2004). Atypical PKC phosphorylates PAR-1 kinases to regulate localization and activity. *Current biology : CB* 14, 736-741.

Huttenlocher, A., and Horwitz, A. R. (2011). Integrins in cell migration. *Cold Spring Harbor perspectives in biology* 3, a005074.

Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell* 110, 673-687.

Iaccarino, I., Hancock, D., Evan, G., and Downward, J. (2003). c-Myc induces cytochrome c release in Rat1 fibroblasts by increasing outer mitochondrial membrane permeability in a Bid-dependent manner. *Cell death and differentiation* 10, 599-608.

Imamura, K., Ogura, T., Kishimoto, A., Kaminishi, M., and Esumi, H. (2001). Cell cycle regulation via p53 phosphorylation by a 5'-AMP activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, in a human hepatocellular carcinoma cell line. *Biochemical and biophysical research communications* 287, 562-567.

Ingman, W. V., and Robertson, S. A. (2008). Mammary gland development in transforming growth factor beta1 null mutant mice: systemic and epithelial effects. *Biology of reproduction* 79, 711-717.

Ingman, W. V., Wyckoff, J., Gouon-Evans, V., Condeelis, J., and Pollard, J. W. (2006). Macrophages promote collagen fibrillogenesis around terminal end buds of the developing mammary gland. *Developmental dynamics : an official publication of the American Association of Anatomists* 235, 3222-3229.

Inoki, K., Zhu, T., and Guan, K. L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115, 577-590.

Iozzo, R. V., and Sanderson, R. D. (2011). Proteoglycans in cancer biology, tumour microenvironment and angiogenesis. *Journal of cellular and molecular medicine* 15, 1013-1031.

Iozzo, R. V., Zoeller, J. J., and Nystrom, A. (2009). Basement membrane proteoglycans: modulators Par Excellence of cancer growth and angiogenesis. *Molecules and cells* 27, 503-513.

Jager, R., Herzer, U., Schenkel, J., and Weiher, H. (1997). Overexpression of Bcl-2 inhibits alveolar cell apoptosis during involution and accelerates c-myc-induced tumorigenesis of the mammary gland in transgenic mice. *Oncogene* 15, 1787-1795.

Jamerson, M. H., Johnson, M. D., Korsmeyer, S. J., Furth, P. A., and Dickson, R. B. (2004). Bax regulates c-Myc-induced mammary tumour apoptosis but not proliferation in MMTV-c-myc transgenic mice. *British journal of cancer* 91, 1372-1379.

Jhappan, C., Geiser, A. G., Kordon, E. C., Bagheri, D., Hennighausen, L., Roberts, A. B., Smith, G. H., and Merlino, G. (1993). Targeting expression of a transforming growth factor beta 1 transgene to the pregnant mammary gland inhibits alveolar development and lactation. *The EMBO journal* 12, 1835-1845.

- Ji, H., Ramsey, M. R., Hayes, D. N., Fan, C., McNamara, K., Kozlowski, P., Torrice, C., Wu, M. C., Shimamura, T., Perera, S. A., *et al.* (2007). LKB1 modulates lung cancer differentiation and metastasis. *Nature* **448**, 807-810.
- Joberty, G., Petersen, C., Gao, L., and Macara, I. G. (2000). The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nature cell biology* **2**, 531-539.
- Johnstone, R. W., Frew, A. J., and Smyth, M. J. (2008). The TRAIL apoptotic pathway in cancer onset, progression and therapy. *Nature reviews Cancer* **8**, 782-798.
- Jones, R. G., Plas, D. R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., Birnbaum, M. J., and Thompson, C. B. (2005). AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Molecular cell* **18**, 283-293.
- Juin, P., Hueber, A. O., Littlewood, T., and Evan, G. (1999). c-Myc-induced sensitization to apoptosis is mediated through cytochrome c release. *Genes & development* **13**, 1367-1381.
- Juin, P., Hunt, A., Littlewood, T., Griffiths, B., Swigart, L. B., Korsmeyer, S., and Evan, G. (2002). c-Myc functionally cooperates with Bax to induce apoptosis. *Molecular and cellular biology* **22**, 6158-6169.
- Junttila, M. R., Puustinen, P., Niemela, M., Ahola, R., Arnold, H., Bottzauw, T., Ala-aho, R., Nielsen, C., Ivaska, J., Taya, Y., *et al.* (2007). CIP2A inhibits PP2A in human malignancies. *Cell* **130**, 51-62.
- Kallay, L. M., McNickle, A., Brennwald, P. J., Hubbard, A. L., and Braiterman, L. T. (2006). Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains. *Journal of cellular biochemistry* **99**, 647-664.
- Kalluri, R. (2003). Basement membranes: structure, assembly and role in tumour angiogenesis. *Nature reviews Cancer* **3**, 422-433.
- Kamalati, T., Niranjan, B., Yant, J., and Buluwela, L. (1999). HGF/SF in mammary epithelial growth and morphogenesis: in vitro and in vivo models. *Journal of mammary gland biology and neoplasia* **4**, 69-77.
- Karp, C. M., Tan, T. T., Mathew, R., Nelson, D., Mukherjee, C., Degenhardt, K., Karantz-Wadsworth, V., and White, E. (2008). Role of the polarity determinant crumbs in suppressing mammalian epithelial tumor progression. *Cancer research* **68**, 4105-4115.
- Karuman, P., Gozani, O., Odze, R. D., Zhou, X. C., Zhu, H., Shaw, R., Brien, T. P., Bozzuto, C. D., Ooi, D., Cantley, L. C., and Yuan, J. (2001). The Peutz-Jegher gene product LKB1 is a mediator of p53-dependent cell death. *Molecular cell* **7**, 1307-1319.
- Katajisto, P., Vaahtomeri, K., Ekman, N., Ventela, E., Ristimäki, A., Bardeesy, N., Feil, R., DePinho, R. A., and Makela, T. P. (2008). LKB1 signaling in mesenchymal cells required for suppression of gastrointestinal polyposis. *Nature genetics* **40**, 455-459.
- Katajisto, P., Vallenius, T., Vaahtomeri, K., Ekman, N., Udd, L., Tiainen, M., and Makela, T. P. (2007). The LKB1 tumor suppressor kinase in human disease. *Biochimica et biophysica acta* **1775**, 63-75.
- Katoh, M., and Katoh, M. (2004). Identification and characterization of human GUKH2 gene in silico. *International journal of oncology* **24**, 1033-1038.
- Kelly, K., Cochran, B. H., Stiles, C. D., and Leder, P. (1983). Cell-specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. *Cell* **35**, 603-610.
- Kelly, P. N., Grabow, S., Delbridge, A. R., Strasser, A., and Adams, J. M. (2011). Endogenous Bcl-xL is essential for Myc-driven lymphomagenesis in mice. *Blood* **118**, 6380-6386.
- Kemphues, K. J., Priess, J. R., Morton, D. G., and Cheng, N. S. (1988). Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* **52**, 311-320.
- Khan, N., Afaq, F., and Mukhtar, H. (2010). Lifestyle as risk factor for cancer: Evidence from human studies. *Cancer letters* **293**, 133-143.
- Kim, B. Y., Yang, J. S., Kwak, S. Y., Zhang, X. K., and Han, Y. H. (2010). NEMO stabilizes c-Myc through direct interaction in the nucleus. *FEBS letters* **584**, 4524-4530.
- Kim, C. J., Cho, Y. G., Park, J. Y., Kim, T. Y., Lee, J. H., Kim, H. S., Lee, J. W., Song, Y. H., Nam, S. W., Lee, S. H., *et al.* (2004). Genetic analysis of the LKB1/STK11 gene in hepatocellular carcinomas. *Eur J Cancer* **40**, 136-141.
- Kim, S., Li, Q., Dang, C. V., and Lee, L. A. (2000a). Induction of ribosomal genes and hepatocyte hypertrophy by adenovirus-mediated expression of c-Myc in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 11198-11202.
- Kim, S. H., Li, Z., and Sacks, D. B. (2000b). E-cadherin-mediated cell-cell attachment activates Cdc42. *The Journal of biological chemistry* **275**, 36999-37005.
- Kimura, T. E., Merritt, A. J., and Garrod, D. R. (2007). Calcium-independent desmosomes of keratinocytes are hyper-adhesive. *The Journal of investigative dermatology* **127**, 775-781.
- Kinzler, K. W., and Vogelstein, B. (1997). Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* **386**, 761, 763.
- Kinzler, K. W., and Vogelstein, B. (1998). Landscaping the cancer terrain. *Science* **280**, 1036-1037.
- Klefstrom, J., Arighi, E., Littlewood, T., Jaattela, M., Saksela, E., Evan, G. I., and Alitalo, K. (1997). Induction of TNF-sensitive cellular phenotype by c-Myc involves p53 and impaired NF-kappaB activation. *The EMBO journal* **16**, 7382-7392.
- Klefstrom, J., Vastrik, I., Saksela, E., Valle, J., Eilers, M., and Alitalo, K. (1994). c-Myc induces cellular susceptibility to the cytotoxic action of TNF-alpha. *The EMBO journal* **13**, 5442-5450.
- Kleine-Kohlbrecher, D., Adhikary, S., and Eilers, M. (2006). Mechanisms of transcriptional repression by Myc. *Current topics in microbiology and immunology* **302**, 51-62.
- Klezovitch, O., Chevillet, J., Mirosevich, J., Roberts, R. L., Matusik, R. J., and Vasioukhin, V. (2004). Hepsin promotes prostate cancer progression and metastasis. *Cancer cell* **6**, 185-195.
- Klinowska, T. C., Alexander, C. M., Georges-Labouesse, E., Van der Neut, R., Kreidberg, J. A., Jones, C. J., Sonnenberg, A., and Streuli, C. H. (2001). Epithelial development and differentiation in the mammary gland is not dependent on alpha 3 or alpha 6 integrin subunits. *Developmental biology* **233**, 449-467.
- Klinowska, T. C., Soriano, J. V., Edwards, G. M., Oliver, J. M., Valentijn, A. J., Montesano, R., and Streuli, C. H. (1999). Laminin and beta1 integrins are crucial for normal mammary gland development in the mouse. *Developmental biology* **215**, 13-32.
- Knoepfler, P. S., Zhang, X. Y., Cheng, P. F., Gafken, P. R., McMahon, S. B., and Eisenman, R. N. (2006). Myc influences global chromatin structure. *The EMBO journal* **25**, 2723-2734.
- Knudson, A. G., Jr. (1971). Mutation and cancer: statistical study of retinoblastoma. *Proceedings of the National Academy of Sciences of the United States of America* **68**, 820-823.

Knust, E., Tepass, U., and Wodarz, A. (1993). crumbs and stardust, two genes of *Drosophila* required for the development of epithelial cell polarity. *Dev Suppl*, 261-268.

Koster, J., Geerts, D., Favre, B., Borradori, L., and Sonnenberg, A. (2003). Analysis of the interactions between BP180, BP230, plectin and the integrin $\alpha 6 \beta 4$ important for hemidesmosome assembly. *Journal of cell science* 116, 387-399.

Kouros-Mehr, H., Slorach, E. M., Sternlicht, M. D., and Werb, Z. (2006). GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. *Cell* 127, 1041-1055.

Kowalczyk, A. P., Stappenbeck, T. S., Parry, D. A., Palka, H. L., Virata, M. L., Bornslaeger, E. A., Nilles, L. A., and Green, K. J. (1994). Structure and function of desmosomal transmembrane core and plaque molecules. *Biophysical chemistry* 50, 97-112.

Krahn, M. P., Buckers, J., Kastrop, L., and Wodarz, A. (2010). Formation of a Bazooka-Stardust complex is essential for plasma membrane polarity in epithelia. *The Journal of cell biology* 190, 751-760.

Kuroda, S., Fukata, M., Fujii, K., Nakamura, T., Izawa, I., and Kaibuchi, K. (1997). Regulation of cell-cell adhesion of MDCK cells by Cdc42 and Rac1 small GTPases. *Biochemical and biophysical research communications* 240, 430-435.

Larsson, L. G., and Henriksson, M. A. (2010). The Yin and Yang functions of the Myc oncoprotein in cancer development and as targets for therapy. *Experimental cell research* 316, 1429-1437.

Lee, E. Y., Lee, W. H., Kaetzel, C. S., Parry, G., and Bissell, M. J. (1985). Interaction of mouse mammary epithelial cells with collagen substrata: regulation of casein gene expression and secretion. *Proceedings of the National Academy of Sciences of the United States of America* 82, 1419-1423.

Lee, E. Y., and Muller, W. J. (2010). Oncogenes and tumor suppressor genes. *Cold Spring Harbor perspectives in biology* 2, a003236.

Lee, J. H., Koh, H., Kim, M., Kim, Y., Lee, S. Y., Karess, R. E., Lee, S. H., Shong, M., Kim, J. M., Kim, J., and Chung, J. (2007). Energy-dependent regulation of cell structure by AMP-activated protein kinase. *Nature* 447, 1017-1020.

Lee, S. S., Glaunsinger, B., Mantovani, F., Banks, L., and Javier, R. T. (2000). Multi-PDZ domain protein MUPP1 is a cellular target for both adenovirus E4-ORF1 and high-risk papillomavirus type 18 E6 oncoproteins. *Journal of virology* 74, 9680-9693.

Legate, K. R., Wickstrom, S. A., and Fassler, R. (2009). Genetic and cell biological analysis of integrin outside-in signaling. *Genes & development* 23, 397-418.

Lemmers, C., Michel, D., Lane-Guermonprez, L., Delgrossi, M. H., Medina, E., Arsanto, J. P., and Le Bivic, A. (2004). CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells. *Molecular biology of the cell* 15, 1324-1333.

Lerga, A., Crespo, P., Berciano, M., Delgado, M. D., Canelles, M., Cales, C., Richard, C., Ceballos, E., Gutierrez, P., Ajenjo, N., *et al.* (1999). Regulation of c-Myc and Max in megakaryocytic and monocytic-macrophagic differentiation of K562 cells induced by protein kinase C modifiers: c-Myc is down-regulated but does not inhibit differentiation. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 10, 639-654.

Leslie, N. R., Batty, I. H., Maccario, H., Davidson, L., and Downes, C. P. (2008). Understanding PTEN regulation: PIP2, polarity and protein stability. *Oncogene* 27, 5464-5476.

Levens, D. (2010). You Don't Muck with MYC. *Genes & cancer* 1, 547-554.

Levental, K. R., Yu, H., Kass, L., Lakins, J. N., Egeblad, M., Erler, J. T., Fong, S. F., Csiszar, K., Giaccia, A., Weninger, W., *et al.* (2009). Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* 139, 891-906.

Li, L. H., Nerlov, C., Prendergast, G., MacGregor, D., and Ziff, E. B. (1994). c-Myc represses transcription in vivo by a novel mechanism dependent on the initiator element and Myc box II. *The EMBO journal* 13, 4070-4079.

Li, M. L., Aggeler, J., Farson, D. A., Hatier, C., Hassell, J., and Bissell, M. J. (1987). Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. *Proceedings of the National Academy of Sciences of the United States of America* 84, 136-140.

Liang, J., Shao, S. H., Xu, Z. X., Hennessy, B., Ding, Z., Larrea, M., Kondo, S., Dumont, D. J., Gutterman, J. U., Walker, C. L., *et al.* (2007). The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. *Nature cell biology* 9, 218-224.

Lin, D., Edwards, A. S., Fawcett, J. P., Mbamalu, G., Scott, J. D., and Pawson, T. (2000). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nature cell biology* 2, 540-547.

Liotta, L. A., Kleinerman, J., Catanzaro, P., and Rynbrandt, D. (1977). Degradation of basement membrane by murine tumor cells. *Journal of the National Cancer Institute* 58, 1427-1431.

Littlewood, T. D., Hancock, D. C., Danielian, P. S., Parker, M. G., and Evan, G. I. (1995). A modified oestrogen receptor ligand-binding domain as an improved switch for the regulation of heterologous proteins. *Nucleic acids research* 23, 1686-1690.

Liu, K., Cheng, L., Flesken-Nikitin, A., Huang, L., Nikitin, A. Y., and Pauli, B. U. (2010). Conditional knockout of fibronectin abrogates mouse mammary gland lobuloalveolar differentiation. *Developmental biology* 346, 11-24.

Liu, K. D., Datta, A., Yu, W., Brakeman, P. R., Jou, T. S., Matthay, M. A., and Mostov, K. E. (2007). Rac1 is required for reorientation of polarity and lumen formation through a PI 3-kinase-dependent pathway. *American journal of physiology Renal physiology* 293, F1633-1640.

Liu, X., Robinson, G. W., Wagner, K. U., Garrett, L., Wynshaw-Boris, A., and Hennighausen, L. (1997). Stat5a is mandatory for adult mammary gland development and lactogenesis. *Genes & development* 11, 179-186.

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25, 402-408.

Lizcano, J. M., Goransson, O., Toth, R., Deak, M., Morrice, N. A., Boudeau, J., Hawley, S. A., Udd, L., Makela, T. P., Hardie, D. G., and Alessi, D. R. (2004). LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *The EMBO journal* 23, 833-843.

Lochter, A., and Bissell, M. J. (1995). Involvement of extracellular matrix constituents in breast cancer. *Seminars in cancer biology* 6, 165-173.

Lochter, A., Galosy, S., Muschler, J., Freedman, N., Werb, Z., and Bissell, M. J. (1997). Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. *The Journal of cell biology* 139, 1861-1872.

Lowe, S. W., Cepero, E., and Evan, G. (2004). Intrinsic tumour suppression. *Nature* 432, 307-315.

Lu, H., and Bilder, D. (2005). Endocytic control of epithelial polarity and proliferation in *Drosophila*. *Nature cell biology* 7, 1232-1239.

Lu, P., Ewald, A. J., Martin, G. R., and Werb, Z. (2008). Genetic mosaic analysis reveals FGF receptor 2 function in terminal end buds during mammary gland branching morphogenesis. *Developmental biology* 321, 77-87.

Lu, P., and Werb, Z. (2008). Patterning mechanisms of branched organs. *Science* 322, 1506-1509.

Lund, L. R., Romer, J., Thomasset, N., Solberg, H., Pyke, C., Bissell, M. J., Dano, K., and Werb, Z. (1996). Two distinct phases of apoptosis in mammary gland involution: proteinase-independent and -dependent pathways. *Development* 122, 181-193.

Luukko, K., Ylikorkala, A., Tiainen, M., and Makela, T. P. (1999). Expression of LKB1 and PTEN tumor suppressor genes during mouse embryonic development. *Mechanisms of development* 83, 187-190.

Lydon, J. P., DeMayo, F. J., Funk, C. R., Mani, S. K., Hughes, A. R., Montgomery, C. A., Jr., Shyamala, G., Conneely, O. M., and O'Malley, B. W. (1995). Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes & development* 9, 2266-2278.

Macara, I. G. (2004). Par proteins: partners in polarization. *Current biology* : CB 14, R160-162.

Maclean, K. H., Keller, U. B., Rodriguez-Galindo, C., Nilsson, J. A., and Cleveland, J. L. (2003). c-Myc augments gamma irradiation-induced apoptosis by suppressing Bcl-XL. *Molecular and cellular biology* 23, 7256-7270.

Macleod, K. (2000). Tumor suppressor genes. *Current opinion in genetics & development* 10, 81-93.

Mailleux, A. A., Overholtzer, M., Schmelzle, T., Bouillet, P., Strasser, A., and Brugge, J. S. (2007). BIM regulates apoptosis during mammary ductal morphogenesis, and its absence reveals alternative cell death mechanisms. *Developmental cell* 12, 221-234.

Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A., and et al. (1990). Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 250, 1233-1238.

Mallepell, S., Krust, A., Chambon, P., and Briskin, C. (2006). Paracrine signaling through the epithelial estrogen receptor alpha is required for proliferation and morphogenesis in the mammary gland. *Proceedings of the National Academy of Sciences of the United States of America* 103, 2196-2201.

Mandai, K., Nakanishi, H., Satoh, A., Obaishi, H., Wada, M., Nishioka, H., Itoh, M., Mizoguchi, A., Aoki, T., Fujimoto, T., et al. (1997). Afadin: A novel actin filament-binding protein with one PDZ domain localized at cadherin-based cell-to-cell adherens junction. *The Journal of cell biology* 139, 517-528.

Marcozzi, C., Burdett, I. D., Buxton, R. S., and Magee, A. I. (1998). Coexpression of both types of desmosomal cadherin and plakoglobin confers strong intercellular adhesion. *Journal of cell science* 111 (Pt 4), 495-509.

Martin, S. G., and St Johnston, D. (2003). A role for *Drosophila* LKB1 in anterior-posterior axis formation and epithelial polarity. *Nature* 421, 379-384.

Martin-Belmonte, F., Gassama, A., Datta, A., Yu, W., Rescher, U., Gerke, V., and Mostov, K. (2007). PTEN-mediated apical segregation of phosphoinositides controls epithelial morphogenesis through Cdc42. *Cell* 128, 383-397.

Martin-Belmonte, F., and Mostov, K. (2008). Regulation of cell polarity during epithelial morphogenesis. *Current opinion in cell biology* 20, 227-234.

Martinou, J. C., and Youle, R. J. (2011). Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Developmental cell* 21, 92-101.

Massey-Harroche, D., Delgrossi, M. H., Lane-Guermonprez, L., Arsanto, J. P., Borg, J. P., Billaud, M., and Le Bivic, A. (2007). Evidence for a molecular link between the tuberous sclerosis complex and the Crumbs complex. *Human molecular genetics* 16, 529-536.

Massimi, P., Narayan, N., Thomas, M., Gammoh, N., Strand, S., Strand, D., and Banks, L. (2008). Regulation of the hDlg/hScrib/Hugl-1 tumour suppressor complex. *Experimental cell research* 314, 3306-3317.

Mastracci, T. L., Tjan, S., Bane, A. L., O'Malley, F. P., and Andrulis, I. L. (2005). E-cadherin alterations in atypical lobular hyperplasia and lobular carcinoma in situ of the breast. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 18, 741-751.

Mateyak, M. K., Obaya, A. J., Adachi, S., and Sedivy, J. M. (1997). Phenotypes of c-Myc-deficient rat fibroblasts isolated by targeted homologous recombination. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 8, 1039-1048.

Mathew, D., Gramates, L. S., Packard, M., Thomas, U., Bilder, D., Perrimon, N., Gorczyca, M., and Budnik, V. (2002). Recruitment of scribble to the synaptic scaffolding complex requires GUK-holder, a novel DLG binding protein. *Current biology* : CB 12, 531-539.

Mazzieri, R., Masiero, L., Zanetta, L., Monea, S., Onisto, M., Garbisa, S., and Mignatti, P. (1997). Control of type IV collagenase activity by components of the urokinase-plasmin system: a regulatory mechanism with cell-bound reactants. *The EMBO journal* 16, 2319-2332.

McCaffrey, L. M., and Macara, I. G. (2009). The Par3/aPKC interaction is essential for end bud remodeling and progenitor differentiation during mammary gland morphogenesis. *Genes & development* 23, 1450-1460.

McCarthy, A., Lord, C. J., Savage, K., Grigoriadis, A., Smith, D. P., Weigelt, B., Reis-Filho, J. S., and Ashworth, A. (2009). Conditional deletion of the Lkb1 gene in the mouse mammary gland induces tumour formation. *The Journal of pathology* 219, 306-316.

McDermott, K. M., Liu, B. Y., Tlsty, T. D., and Pazour, G. J. (2010). Primary cilia regulate branching morphogenesis during mammary gland development. *Current biology* : CB 20, 731-737.

McLachlan, E., Shao, Q., and Laird, D. W. (2007). Connexins and gap junctions in mammary gland development and breast cancer progression. *The Journal of membrane biology* 218, 107-121.

McMahon, S. B., Van Buskirk, H. A., Dugan, K. A., Copeland, T. D., and Cole, M. D. (1998). The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. *Cell* 94, 363-374.

McMahon, S. B., Wood, M. A., and Cole, M. D. (2000). The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. *Molecular and cellular biology* 20, 556-562.

Mehenni, H., Resta, N., Park, J. G., Miyaki, M., Guanti, G., and Costanza, M. C. (2006). Cancer risks in LKB1 germline mutation carriers. *Gut* 55, 984-990.

Menut, L., Vaccari, T., Dionne, H., Hill, J., Wu, G., and Bilder, D. (2007). A mosaic genetic screen for *Drosophila* neoplastic tumor suppressor genes based on defective pupation. *Genetics* 177, 1667-1677.

Metcalfe, A. D., Gilmore, A., Klinowska, T., Oliver, J., Valentijn, A. J., Brown, R., Ross, A., MacGregor, G., Hickman, J. A., and Streuli, C. H. (1999). Developmental regulation of Bcl-2 family protein expression in the involuting mammary gland. *Journal of cell science* 112 (Pt 11), 1771-1783.

Meyer, N., and Penn, L. Z. (2008). Reflecting on 25 years with MYC. *Nature reviews Cancer* 8, 976-990.

Miao, J., Mu, D., Ergel, B., Singavarapu, R., Duan, Z., Powers, S., Oliva, E., and Orsulic, S. (2008). Hepsin colocalizes with desmosomes and induces progression of ovarian cancer in a mouse model. *International journal of cancer Journal international du cancer* 123, 2041-2047.

Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., Ding, W., and et al. (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266, 66-71.

Miravet, S., Piedra, J., Miro, F., Itarte, E., Garcia de Herreros, A., and Dunach, M. (2002). The transcriptional factor Tcf-4 contains different binding sites for beta-catenin and plakoglobin. *The Journal of biological chemistry* 277, 1884-1891.

Mirouse, V., Swick, L. L., Kazgan, N., St Johnston, D., and Brenman, J. E. (2007). LKB1 and AMPK maintain epithelial cell polarity under energetic stress. *The Journal of cell biology* 177, 387-392.

Mitchell, K. O., Ricci, M. S., Miyashita, T., Dicker, D. T., Jin, Z., Reed, J. C., and El-Deiry, W. S. (2000). Bax is a transcriptional target and mediator of c-myc-induced apoptosis. *Cancer research* 60, 6318-6325.

Miyashita, T., Krajewski, S., Krajewska, M., Wang, H. G., Lin, H. K., Liebermann, D. A., Hoffman, B., and Reed, J. C. (1994). Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene* 9, 1799-1805.

Miyashita, T., and Reed, J. C. (1995). Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 80, 293-299.

Moberg, K. H., Schelble, S., Burdick, S. K., and Hariharan, I. K. (2005). Mutations in erupted, the Drosophila ortholog of mammalian tumor susceptibility gene 101, elicit non-cell-autonomous overgrowth. *Developmental cell* 9, 699-710.

Mongiat, B., Taylor, K., Otto, J., Aho, S., Uitto, J., Whitelock, J. M., and Iozzo, R. V. (2000). The protein core of the proteoglycan perlecan binds specifically to fibroblast growth factor-7. *The Journal of biological chemistry* 275, 7095-7100.

Morais-de-Sa, E., Mirouse, V., and St Johnston, D. (2010). aPKC phosphorylation of Bazooka defines the apical/lateral border in Drosophila epithelial cells. *Cell* 141, 509-523.

Morgan, M. R., Humphries, M. J., and Bass, M. D. (2007). Synergistic control of cell adhesion by integrins and syndecans. *Nature reviews Molecular cell biology* 8, 957-969.

Morton, D. G., Shakes, D. C., Nugent, S., Dichoso, D., Wang, W., Golden, A., and Kempthues, K. J. (2002). The Caenorhabditis elegans par-5 gene encodes a 14-3-3 protein required for cellular asymmetry in the early embryo. *Developmental biology* 241, 47-58.

Morton, J. P., Jamieson, N. B., Karim, S. A., Athineos, D., Ridgway, R. A., Nixon, C., McKay, C. J., Carter, R., Brunton, V. G., Frame, M. C., et al. (2010). LKB1 haploinsufficiency cooperates with Kras to promote pancreatic cancer through suppression of p21-dependent growth arrest. *Gastroenterology* 139, 586-597, 597 e581-586.

Murphy, D. J., Junttila, M. R., Pouyet, L., Karnezis, A., Shchors, K., Bui, D. A., Brown-Swigart, L., Johnson, L., and Evan, G. I. (2008). Distinct thresholds govern Myc's biological output in vivo. *Cancer cell* 14, 447-457.

Murray, N. R., Jamieson, L., Yu, W., Zhang, J., Gokmen-Polar, Y., Sier, D., Anastasiadis, P., Gatalica, Z., Thompson, E. A., and Fields, A. P. (2004). Protein kinase Ciot is required for Ras transformation and colon carcinogenesis in vivo. *The Journal of cell biology* 164, 797-802.

Muschler, J., and Streuli, C. H. (2010). Cell-matrix interactions in mammary gland development and breast cancer. *Cold Spring Harbor perspectives in biology* 2, a003202.

Muthuswamy, S. K., Li, D., Lelievre, S., Bissell, M. J., and Brugge, J. S. (2001). ErbB2, but not ErbB1, reinitiates proliferation and induces luminal repopulation in epithelial acini. *Nature cell biology* 3, 785-792.

Myllymaki, S. M., Teravainen, T. P., and Manninen, A. (2011). Two distinct integrin-mediated mechanisms contribute to apical lumen formation in epithelial cells. *PloS one* 6, e19453.

Naidu, R., Wahab, N. A., Yadav, M., and Kutty, M. K. (2002). Protein expression and molecular analysis of c-myc gene in primary breast carcinomas using immunohistochemistry and differential polymerase chain reaction. *International journal of molecular medicine* 9, 189-196.

Nakagawa, S., and Huijbregtse, J. M. (2000). Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase. *Molecular and cellular biology* 20, 8244-8253.

Nakau, M., Miyoshi, H., Seldin, M. F., Imamura, M., Oshima, M., and Taketo, M. M. (2002). Hepatocellular carcinoma caused by loss of heterozygosity in Lkb1 gene knockout mice. *Cancer research* 62, 4549-4553.

Nakaya, Y., Sukowati, E. W., Wu, Y., and Sheng, G. (2008). RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. *Nature cell biology* 10, 765-775.

Nandana, S., Ellwood-Yen, K., Sawyers, C., Wills, M., Weidow, B., Case, T., Vasioukhin, V., and Matusik, R. (2010). Hepsin cooperates with MYC in the progression of adenocarcinoma in a prostate cancer mouse model. *The Prostate* 70, 591-600.

Nathanson, K. L., Wooster, R., and Weber, B. L. (2001). Breast cancer genetics: what we know and what we need. *Nature medicine* 7, 552-556.

Navarro, C., Nola, S., Audebert, S., Santoni, M. J., Arsanto, J. P., Ginestier, C., Marchetto, S., Jacquemier, J., Isnardon, D., Le Bivic, A., et al. (2005). Junctional recruitment of mammalian Scribble relies on E-cadherin engagement. *Oncogene* 24, 4330-4339.

Naylor, M. J., Li, N., Cheung, J., Lowe, E. T., Lambert, E., Marlow, R., Wang, P., Schatzmann, F., Wintermantel, T., Schuetz, G., et al. (2005a). Ablation of beta1 integrin in mammary epithelium reveals a key role for integrin in glandular morphogenesis and differentiation. *The Journal of cell biology* 171, 717-728.

Naylor, M. J., Oakes, S. R., Gardiner-Garden, M., Harris, J., Blazek, K., Ho, T. W., Li, F. C., Wynick, D., Walker, A. M., and Ormandy, C. J. (2005b). Transcriptional changes underlying the secretory activation phase of mammary gland development. *Mol Endocrinol* 19, 1868-1883.

Neville, M. C., McFadden, T. B., and Forsyth, I. (2002). Hormonal regulation of mammary differentiation and milk secretion. *Journal of mammary gland biology and neoplasia* 7, 49-66.

Nezu, J., Oku, A., and Shimane, M. (1999). Loss of cytoplasmic retention ability of mutant LKB1 found in Peutz-Jeghers syndrome patients. *Biochemical and biophysical research communications* 261, 750-755.

Nieminen, A. I., Partanen, J. I., and Klefstrom, J. (2007). c-Myc blazing a trail of death: coupling of the mitochondrial and death receptor apoptosis pathways by c-Myc. *Cell Cycle* 6, 2464-2472.

Noda, Y., Takeya, R., Ohno, S., Naito, S., Ito, T., and Sumimoto, H. (2001). Human homologues of the *Caenorhabditis elegans* cell polarity protein PAR6 as an adaptor that links the small GTPases Rac and Cdc42 to atypical protein kinase C. *Genes to cells : devoted to molecular & cellular mechanisms* 6, 107-119.

Nolan, M. E., Aranda, V., Lee, S., Lakshmi, B., Basu, S., Allred, D. C., and Muthuswamy, S. K. (2008). The polarity protein Par6 induces cell proliferation and is overexpressed in breast cancer. *Cancer research* 68, 8201-8209.

Noren, N. K., Niessen, C. M., Gumbiner, B. M., and Burridge, K. (2001). Cadherin engagement regulates Rho family GTPases. *The Journal of biological chemistry* 276, 33305-33308.

O'Brien, L. E., Jou, T. S., Pollack, A. L., Zhang, Q., Hansen, S. H., Yurchenco, P., and Mostov, K. E. (2001). Rac1 orientates epithelial apical polarity through effects on basolateral laminin assembly. *Nature cell biology* 3, 831-838.

Oda, E., Ohki, R., Murasawa, H., Nemoto, J., Shibue, T., Yamashita, T., Tokino, T., Taniguchi, T., and Tanaka, N. (2000). Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* 288, 1053-1058.

Ogura, Y., Matsunaga, Y., Nishiyama, T., and Amano, S. (2008). Plasmin induces degradation and dysfunction of laminin 332 (laminin 5) and impaired assembly of basement membrane at the dermal-epidermal junction. *The British journal of dermatology* 159, 49-60.

Oliveira, A. M., Ross, J. S., and Fletcher, J. A. (2005). Tumor suppressor genes in breast cancer: the gatekeepers and the caretakers. *American journal of clinical pathology* 124 Suppl, S16-28.

Ollila, S., and Makela, T. P. (2011). The tumor suppressor kinase LKB1: lessons from mouse models. *Journal of molecular cell biology* 3, 330-340.

Opper, M., Schuler, G., and Mechler, B. M. (1987). Hereditary suppression of lethal (2) giant larvae malignant tumor development in *Drosophila* by gene transfer. *Oncogene* 1, 91-96.

Orlando, K., and Guo, W. (2009). Membrane organization and dynamics in cell polarity. *Cold Spring Harbor perspectives in biology* 1, a001321.

Ormandy, C. J., Binart, N., and Kelly, P. A. (1997). Mammary gland development in prolactin receptor knockout mice. *Journal of mammary gland biology and neoplasia* 2, 355-364.

Owen, K. A., Qiu, D., Alves, J., Schumacher, A. M., Kilpatrick, L. M., Li, J., Harris, J. L., and Ellis, V. (2010). Pericellular activation of hepatocyte growth factor by the transmembrane serine proteases matriptase and hepsin, but not by the membrane-associated protease uPA. *The Biochemical journal* 426, 219-228.

Page-McCaw, A., Ewald, A. J., and Werb, Z. (2007). Matrix metalloproteinases and the regulation of tissue remodelling. *Nature reviews Molecular cell biology* 8, 221-233.

Pagliarini, R. A., and Xu, T. (2003). A genetic screen in *Drosophila* for metastatic behavior. *Science* 302, 1227-1231.

Parmar, H., and Cunha, G. R. (2004). Epithelial-stromal interactions in the mouse and human mammary gland in vivo. *Endocrine-related cancer* 11, 437-458.

Parsa, S., Ramasamy, S. K., De Langhe, S., Gupte, V. V., Haigh, J. J., Medina, D., and Bellusci, S. (2008). Terminal end bud maintenance in mammary gland is dependent upon FGFR2b signaling. *Developmental biology* 317, 121-131.

Pauklin, S., Kristjuhan, A., Maimets, T., and Jaks, V. (2005). ARF and ATM/ATR cooperate in p53-mediated apoptosis upon oncogenic stress. *Biochemical and biophysical research communications* 334, 386-394.

Payne, S. R., and Kemp, C. J. (2005). Tumor suppressor genetics. *Carcinogenesis* 26, 2031-2045.

Pelengaris, S., Khan, M., and Evan, G. (2002a). c-MYC: more than just a matter of life and death. *Nature reviews Cancer* 2, 764-776.

Pelengaris, S., Khan, M., and Evan, G. I. (2002b). Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell* 109, 321-334.

Pierce, D. F., Jr., Johnson, M. D., Matsui, Y., Robinson, S. D., Gold, L. I., Purchio, A. F., Daniel, C. W., Hogan, B. L., and Moses, H. L. (1993). Inhibition of mammary duct development but not alveolar outgrowth during pregnancy in transgenic mice expressing active TGF-beta 1. *Genes & development* 7, 2308-2317.

Pietras, K., and Ostman, A. (2010). Hallmarks of cancer: interactions with the tumor stroma. *Experimental cell research* 316, 1324-1331.

Pitelka, D. R., Hamamoto, S. T., Duafala, J. G., and Nemanic, M. K. (1973). Cell contacts in the mouse mammary gland. I. Normal gland in postnatal development and the secretory cycle. *The Journal of cell biology* 56, 797-818.

Plum, A., Hallas, G., Magin, T., Dombrowski, F., Hagendorff, A., Schumacher, B., Wolpert, C., Kim, J., Lamers, W. H., Evert, M., et al. (2000). Unique and shared functions of different connexins in mice. *Current biology : CB* 10, 1083-1091.

Polyak, K., and Kalluri, R. (2010). The role of the microenvironment in mammary gland development and cancer. *Cold Spring Harbor perspectives in biology* 2, a003244.

Prochownik, E. V., and Kukowska, J. (1986). Deregulated expression of c-myc by murine erythroleukaemia cells prevents differentiation. *Nature* 322, 848-850.

Pullan, S., Wilson, J., Metcalfe, A., Edwards, G. M., Goberdhan, N., Tilly, J., Hickman, J. A., Dive, C., and Streuli, C. H. (1996). Requirement of basement membrane for the suppression of programmed cell death in mammary epithelium. *Journal of cell science* 109 (Pt 3), 631-642.

Pulverer, B. J., Fisher, C., Vousden, K., Littlewood, T., Evan, G., and Woodgett, J. R. (1994). Site-specific modulation of c-Myc cotransformation by residues phosphorylated in vivo. *Oncogene* 9, 59-70.

Pusapati, R. V., Rounbehler, R. J., Hong, S., Powers, J. T., Yan, M., Kiguchi, K., McArthur, M. J., Wong, P. K., and Johnson, D. G. (2006). ATM promotes apoptosis and suppresses tumorigenesis in response to Myc. *Proceedings of the National Academy of Sciences of the United States of America* 103, 1446-1451.

Queva, C., Hurlin, P. J., Foley, K. P., and Eisenman, R. N. (1998). Sequential expression of the MAD family of transcriptional repressors during differentiation and development. *Oncogene* 16, 967-977.

Rahl, P. B., Lin, C. Y., Seila, A. C., Flynn, R. A., McCuine, S., Burge, C. B., Sharp, P. A., and Young, R. A. (2010). c-Myc regulates transcriptional pause release. *Cell* 141, 432-445.

Rakha, E. A., Abd El Rehim, D., Pinder, S. E., Lewis, S. A., and Ellis, I. O. (2005). E-cadherin expression in invasive non-lobular carcinoma of the breast and its prognostic significance. *Histopathology* 46, 685-693.

Reeves, G. K., Pirie, K., Green, J., Bull, D., and Beral, V. (2011). Comparison of the effects of genetic and environmental risk factors on in situ and invasive ductal breast cancer. *International journal of cancer Journal international du cancer*.

Regala, R. P., Weems, C., Jamieson, L., Khoor, A., Edell, E. S., Lohse, C. M., and Fields, A. P. (2005). Atypical protein kinase C ι is an oncogene in human non-small cell lung cancer. *Cancer research* 65, 8905-8911.

Reginato, M. J., Mills, K. R., Becker, E. B., Lynch, D. K., Bonni, A., Muthuswamy, S. K., and Brugge, J. S. (2005). Bim regulation of lumen formation in cultured mammary epithelial acini is targeted by oncogenes. *Molecular and cellular biology* 25, 4591-4601.

Reginato, M. J., Mills, K. R., Paulus, J. K., Lynch, D. K., Sgroi, D. C., Debnath, J., Muthuswamy, S. K., and Brugge, J. S. (2003). Integrins and EGFR coordinately regulate the pro-apoptotic protein Bim to prevent anoikis. *Nature cell biology* 5, 733-740.

Ricci, M. S., Jin, Z., Dewes, M., Yu, D., Thomas-Tikhonenko, A., Dicker, D. T., and El-Deiry, W. S. (2004). Direct repression of FLIP expression by c-myc is a major determinant of TRAIL sensitivity. *Molecular and cellular biology* 24, 8541-8555.

Ricci, M. S., Kim, S. H., Ogi, K., Plastaras, J. P., Ling, J., Wang, W., Jin, Z., Liu, Y. Y., Dicker, D. T., Chiao, P. J., *et al.* (2007). Reduction of TRAIL-induced Mcl-1 and cIAP2 by c-Myc or sorafenib sensitizes resistant human cancer cells to TRAIL-induced death. *Cancer cell* 12, 66-80.

Richert, M. M., Schwertfeger, K. L., Ryder, J. W., and Anderson, S. M. (2000). An atlas of mouse mammary gland development. *Journal of mammary gland biology and neoplasia* 5, 227-241.

Ripperger, T., Gadzicki, D., Meindl, A., and Schlegelberger, B. (2009). Breast cancer susceptibility: current knowledge and implications for genetic counselling. *European journal of human genetics* : EJHG 17, 722-731.

Robinson, G. W., McKnight, R. A., Smith, G. H., and Hennighausen, L. (1995). Mammary epithelial cells undergo secretory differentiation in cycling virgins but require pregnancy for the establishment of terminal differentiation. *Development* 121, 2079-2090.

Roh, M. H., Fan, S., Liu, C. J., and Margolis, B. (2003). The Crumbs3-Pals1 complex participates in the establishment of polarity in mammalian epithelial cells. *Journal of cell science* 116, 2895-2906.

Rothenberg, S. M., Mohapatra, G., Rivera, M. N., Winokur, D., Greninger, P., Nitta, M., Sadow, P. M., Sooriyakumar, G., Brannigan, B. W., Ulman, M. J., *et al.* (2010). A genome-wide screen for microdeletions reveals disruption of polarity complex genes in diverse human cancers. *Cancer research* 70, 2158-2164.

Rowan, A., Churchman, M., Jefferey, R., Hanby, A., Poulson, R., and Tomlinson, I. (2000). In situ analysis of LKB1/STK11 mRNA expression in human normal tissues and tumours. *The Journal of pathology* 192, 203-206.

Rummukainen, J. K., Salminen, T., Lundin, J., Kytola, S., Joensuu, H., and Isola, J. J. (2001). Amplification of c-myc by fluorescence in situ hybridization in a population-based breast cancer tissue array. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 14, 1030-1035.

Saadat, I., Higashi, H., Obuse, C., Umeda, M., Murata-Kamiya, N., Saito, Y., Lu, H., Ohnishi, N., Azuma, T., Suzuki, A., *et al.* (2007). *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. *Nature* 447, 330-333.

Saal, L. H., Gruvberger-Saal, S. K., Persson, C., Lovgren, K., Jumppanen, M., Staaf, J., Jonsson, G., Pires, M. M., Maurer, M., Holm, K., *et al.* (2008). Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. *Nature genetics* 40, 102-107.

Sahin, F., Maitra, A., Argani, P., Sato, N., Maehara, N., Montgomery, E., Goggins, M., Hruban, R. H., and Su, G. H. (2003). Loss of Stk11/Lkb1 expression in pancreatic and biliary neoplasms. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 16, 686-691.

Sakamuro, D., Eviner, V., Elliott, K. J., Showe, L., White, E., and Prendergast, G. C. (1995). c-Myc induces apoptosis in epithelial cells by both p53-dependent and p53-independent mechanisms. *Oncogene* 11, 2411-2418.

Sanchez-Cespedes, M., Parrella, P., Esteller, M., Nomoto, S., Trink, B., Engles, J. M., Westra, W. H., Herman, J. G., and Sidransky, D. (2002). Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. *Cancer research* 62, 3659-3662.

Sandgren, E. P., Schroeder, J. A., Qui, T. H., Palmiter, R. D., Brinster, R. L., and Lee, D. C. (1995). Inhibition of mammary gland involution is associated with transforming growth factor alpha but not c-myc-induced tumorigenesis in transgenic mice. *Cancer research* 55, 3915-3927.

Santos, S. J., Haslam, S. Z., and Conrad, S. E. (2010). Signal transducer and activator of transcription 5a mediates mammary ductal branching and proliferation in the nulliparous mouse. *Endocrinology* 151, 2876-2885.

Sarkisian, C. J., Keister, B. A., Stairs, D. B., Boxer, R. B., Moody, S. E., and Chodosh, L. A. (2007). Dose-dependent oncogene-induced senescence in vivo and its evasion during mammary tumorigenesis. *Nature cell biology* 9, 493-505.

Schaapveld, R. Q., Borradori, L., Geerts, D., van Leusden, M. R., Kuikman, I., Nievers, M. G., Niessen, C. M., Steenbergen, R. D., Snijders, P. J., and Sonnenberg, A. (1998). Hemidesmosome formation is initiated by the beta4 integrin subunit, requires complex formation of beta4 and HD1/plectin, and involves a direct interaction between beta4 and the bullous pemphigoid antigen 180. *The Journal of cell biology* 142, 271-284.

Schoenenberger, C. A., Andres, A. C., Groner, B., van der Valk, M., LeMeur, M., and Gerlinger, P. (1988). Targeted c-myc gene expression in mammary glands of transgenic mice induces mammary tumours with constitutive milk protein gene transcription. *The EMBO journal* 7, 169-175.

Sears, R., Nuckolls, F., Haura, E., Taya, Y., Tamai, K., and Nevins, J. R. (2000). Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes & development* 14, 2501-2514.

Sebbagh, M., Santoni, M. J., Hall, B., Borg, J. P., and Schwartz, M. A. (2009). Regulation of LKB1/STK11 localization and function by E-cadherin. *Current biology* : CB 19, 37-42.

Seoane, J., Le, H. V., and Massague, J. (2002). Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. *Nature* 419, 729-734.

Shackelford, D. B., and Shaw, R. J. (2009). The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nature reviews Cancer* 9, 563-575.

Shackleton, M., Vaillant, F., Simpson, K. J., Stingl, J., Smyth, G. K., Asselin-Labat, M. L., Wu, L., Lindeman, G. J., and Visvader, J. E. (2006). Generation of a functional mammary gland from a single stem cell. *Nature* 439, 84-88.

Shaw, R. J., Kosmatka, M., Bardeesy, N., Hurley, R. L., Witters, L. A., DePinho, R. A., and Cantley, L. C. (2004). The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proceedings of the National Academy of Sciences of the United States of America* 101, 3329-3335.

Sheiness, D., and Bishop, J. M. (1979). DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. *Journal of virology* *31*, 514-521.

Shelly, M., Cancedda, L., Heilshorn, S., Sumbre, G., and Poo, M. M. (2007). LKB1/STRAD promotes axon initiation during neuronal polarization. *Cell* *129*, 565-577.

Shen, Z., Wen, X. F., Lan, F., Shen, Z. Z., and Shao, Z. M. (2002). The tumor suppressor gene LKB1 is associated with prognosis in human breast carcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research* *8*, 2085-2090.

Shin, K., Fogg, V. C., and Margolis, B. (2006). Tight junctions and cell polarity. *Annual review of cell and developmental biology* *22*, 207-235.

Shin, K., Straight, S., and Margolis, B. (2005). PATJ regulates tight junction formation and polarity in mammalian epithelial cells. *The Journal of cell biology* *168*, 705-711.

Siegel, P. M., Shu, W., Cardiff, R. D., Muller, W. J., and Massague, J. (2003). Transforming growth factor beta signaling impairs Neu-induced mammary tumorigenesis while promoting pulmonary metastasis. *Proceedings of the National Academy of Sciences of the United States of America* *100*, 8430-8435.

Silberstein, G. B., and Daniel, C. W. (1982). Glycosaminoglycans in the basal lamina and extracellular matrix of the developing mouse mammary duct. *Developmental biology* *90*, 215-222.

Silberstein, G. B., Flanders, K. C., Roberts, A. B., and Daniel, C. W. (1992). Regulation of mammary morphogenesis: evidence for extracellular matrix-mediated inhibition of ductal budding by transforming growth factor-beta 1. *Developmental biology* *152*, 354-362.

Simian, M., Hirai, Y., Navre, M., Werb, Z., Lochter, A., and Bissell, M. J. (2001). The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. *Development* *128*, 3117-3131.

Sinn, E., Muller, W., Pattengale, P., Tepler, I., Wallace, R., and Leder, P. (1987). Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. *Cell* *49*, 465-475.

Sjoblom, T., Jones, S., Wood, L. D., Parsons, D. W., Lin, J., Barber, T. D., Mandelker, D., Leary, R. J., Ptak, J., Silliman, N., *et al.* (2006). The consensus coding sequences of human breast and colorectal cancers. *Science* *314*, 268-274.

Sleeman, K. E., Kendrick, H., Ashworth, A., Isacke, C. M., and Smalley, M. J. (2006). CD24 staining of mouse mammary gland cells defines luminal epithelial, myoepithelial/basal and non-epithelial cells. *Breast cancer research : BCR* *8*, R7.

Sorlie, T., Perou, C. M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., *et al.* (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proceedings of the National Academy of Sciences of the United States of America* *98*, 10869-10874.

Sorrosal, G., Perez, L., Herranz, H., and Milan, M. (2010). Scarface, a secreted serine protease-like protein, regulates polarized localization of laminin A at the basement membrane of the Drosophila embryo. *EMBO reports* *11*, 373-379.

Sotillos, S., Diaz-Meco, M. T., Caminero, E., Moscat, J., and Campuzano, S. (2004). DaPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in Drosophila. *The Journal of cell biology* *166*, 549-557.

Staller, P., Peukert, K., Kiermaier, A., Seoane, J., Lukas, J., Karsunky, H., Moroy, T., Bartek, J., Massague, J., Hanel, F., and Eilers, M. (2001). Repression of p15INK4b expression by Myc through association with Miz-1. *Nature cell biology* *3*, 392-399.

Steffgen, K., Dufraux, K., and Hathaway, H. (2002). Enhanced branching morphogenesis in mammary glands of mice lacking cell surface beta1,4-galactosyltransferase. *Developmental biology* *244*, 114-133.

Sternlicht, M. D., Kouros-Mehr, H., Lu, P., and Werb, Z. (2006). Hormonal and local control of mammary branching morphogenesis. *Differentiation; research in biological diversity* *74*, 365-381.

Sternlicht, M. D., and Werb, Z. (2001). How matrix metalloproteinases regulate cell behavior. *Annual review of cell and developmental biology* *17*, 463-516.

Stewart, T. A., Pattengale, P. K., and Leder, P. (1984). Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell* *38*, 627-637.

Stingl, J., Eirew, P., Ricketson, I., Shackleton, M., Vaillant, F., Choi, D., Li, H. I., and Eaves, C. J. (2006). Purification and unique properties of mammary epithelial stem cells. *Nature* *439*, 993-997.

Storrs, C. H., and Silverstein, S. J. (2007). PATJ, a tight junction-associated PDZ protein, is a novel degradation target of high-risk human papillomavirus E6 and the alternatively spliced isoform 18 E6. *Journal of virology* *81*, 4080-4090.

Straight, S. W., Shin, K., Fogg, V. C., Fan, S., Liu, C. J., Roh, M., and Margolis, B. (2004). Loss of PALS1 expression leads to tight junction and polarity defects. *Molecular biology of the cell* *15*, 1981-1990.

Streuli, C. H., Bailey, N., and Bissell, M. J. (1991). Control of mammary epithelial differentiation: basement membrane induces tissue-specific gene expression in the absence of cell-cell interaction and morphological polarity. *The Journal of cell biology* *115*, 1383-1395.

Streuli, C. H., Schmidhauser, C., Bailey, N., Yurchenco, P., Skubitz, A. P., Roskelley, C., and Bissell, M. J. (1995). Laminin mediates tissue-specific gene expression in mammary epithelia. *The Journal of cell biology* *129*, 591-603.

Su, G. H., Hruban, R. H., Bansal, R. K., Bova, G. S., Tang, D. J., Shekher, M. C., Westerman, A. M., Entius, M. M., Goggins, M., Yeo, C. J., and Kern, S. E. (1999). Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. *The American journal of pathology* *154*, 1835-1840.

Suzuki, A., Hirata, M., Kamimura, K., Maniwa, R., Yamanaka, T., Mizuno, K., Kishikawa, M., Hirose, H., Amano, Y., Izumi, N., *et al.* (2004). aPKC acts upstream of PAR-1b in both the establishment and maintenance of mammalian epithelial polarity. *Current biology : CB* *14*, 1425-1435.

Suzuki, A., and Ohno, S. (2006). The PAR-aPKC system: lessons in polarity. *Journal of cell science* *119*, 979-987.

Sympson, C. J., Talhouk, R. S., Alexander, C. M., Chin, J. R., Clift, S. M., Bissell, M. J., and Werb, Z. (1994). Targeted expression of stromelysin-1 in mammary gland provides evidence for a role of proteinases in branching morphogenesis and the requirement for an intact basement membrane for tissue-specific gene expression. *The Journal of cell biology* *125*, 681-693.

Szczepanska, K., and Maleszewski, M. (2005). LKB1/PAR4 protein is asymmetrically localized in mouse oocytes and associates with meiotic spindle. *Gene expression patterns : GEP* *6*, 86-93.

Taddei, I., Deugnier, M. A., Faraldo, M. M., Petit, V., Bouvard, D., Medina, D., Fassler, R., Thiery, J. P., and Glukhova, M. A. (2008). Beta1 integrin deletion from the basal compartment of the mammary epithelium affects stem cells. *Nature cell biology* *10*, 716-722.

Taddei, I., Faraldo, M. M., Teuliere, J., Deugnier, M. A., Thiery, J. P., and Glukhova, M. A. (2003). Integrins in mammary gland development and differentiation of mammary epithelium. *Journal of mammary gland biology and neoplasia* *8*, 383-394.

Takahama, S., Hirose, T., and Ohno, S. (2008). aPKC restricts the basolateral determinant PtdIns(3,4,5)P₃ to the basal region. *Biochemical and biophysical research communications* *368*, 249-255.

Takahashi, K., Nakanishi, H., Miyahara, M., Mandai, K., Satoh, K., Satoh, A., Nishioka, H., Aoki, J., Nomoto, A., Mizoguchi, A., and Takai, Y. (1999). Nectin/PRR: an immunoglobulin-like cell adhesion molecule recruited to cadherin-based adherens junctions through interaction with Afadin, a PDZ domain-containing protein. *The Journal of cell biology* *145*, 539-549.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* *131*, 861-872.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* *126*, 663-676.

Tapon, N., Ito, N., Dickson, B. J., Treisman, J. E., and Hariharan, I. K. (2001). The Drosophila tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. *Cell* *105*, 345-355.

Tassan, J. P., and Le Goff, X. (2004). An overview of the KIN1/Par-1/MARK kinase family. *Biology of the cell / under the auspices of the European Cell Biology Organization* *96*, 193-199.

Tervonen, T. A., Partanen, J. I., Saarikoski, S. T., Myllynen, M., Marques, E., Paasonen, K., Moilanen, A., Wohlfahrt, G., Kovanen, P. E., and Klefstrom, J. (2011). Faulty epithelial polarity genes and cancer. *Advances in cancer research* *111*, 97-161.

Thompson, C. B., Challoner, P. B., Neiman, P. E., and Groudine, M. (1985). Levels of c-myc oncogene mRNA are invariant throughout the cell cycle. *Nature* *314*, 363-366.

Tiainen, M., Vaahtomeri, K., Ylikorkala, A., and Makela, T. P. (2002). Growth arrest by the LKB1 tumor suppressor: induction of p21(WAF1/CIP1). *Human molecular genetics* *11*, 1497-1504.

Tian, A. G., and Deng, W. M. (2008). Lgl and its phosphorylation by aPKC regulate oocyte polarity formation in Drosophila. *Development* *135*, 463-471.

Tian, A. G., and Deng, W. M. (2009). Par-1 and Tau regulate the anterior-posterior gradient of microtubules in Drosophila oocytes. *Developmental biology* *327*, 458-464.

Tripathi, M., Nandana, S., Yamashita, H., Ganesan, R., Kirchhofer, D., and Quaranta, V. (2008). Laminin-332 is a substrate for hepsin, a protease associated with prostate cancer progression. *The Journal of biological chemistry* *283*, 30576-30584.

Trumpp, A., Refaeli, Y., Oskarsson, T., Gasser, S., Murphy, M., Martin, G. R., and Bishop, J. M. (2001). c-Myc regulates mammalian body size by controlling cell number but not cell size. *Nature* *414*, 768-773.

Tsai, J. H., Hsieh, Y. S., Kuo, S. J., Chen, S. T., Yu, S. Y., Huang, C. Y., Chang, A. C., Wang, Y. W., Tsai, M. T., and Liu, J. Y. (2000). Alteration in the expression of protein kinase C isoforms in human hepatocellular carcinoma. *Cancer letters* *161*, 171-175.

Udd, L., Katajisto, P., Kyyronen, M., Ristimäki, A. P., and Makela, T. P. (2010). Impaired gastric gland differentiation in Peutz-Jeghers syndrome. *The American journal of pathology* *176*, 2467-2476.

Umeda, K., Ikenouchi, J., Katahira-Tayama, S., Furuse, K., Sasaki, H., Nakayama, M., Matsui, T., Tsukita, S., Furuse, M., and Tsukita, S. (2006). ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. *Cell* *126*, 741-754.

Underwood, J. M., Imbalzano, K. M., Weaver, V. M., Fischer, A. H., Imbalzano, A. N., and Nickerson, J. A. (2006). The ultrastructure of MCF-10A acini. *Journal of cellular physiology* *208*, 141-148.

Vaahtomeri, K., Ventela, E., Laajanen, K., Katajisto, P., Wipff, P. J., Hinz, B., Vallenius, T., Tiainen, M., and Makela, T. P. (2008). Lkb1 is required for TGFbeta-mediated myofibroblast differentiation. *Journal of cell science* *121*, 3531-3540.

Vaccari, T., and Bilder, D. (2005). The Drosophila tumor suppressor vps25 prevents nonautonomous overproliferation by regulating notch trafficking. *Developmental cell* *9*, 687-698.

Vafaizadeh, V., Klemmt, P., Brendel, C., Weber, K., Doebele, C., Britt, K., Grez, M., Fehse, B., Desrivieres, S., and Groner, B. (2010). Mammary epithelial reconstitution with gene-modified stem cells assigns roles to Stat5 in luminal alveolar cell fate decisions, differentiation, involution, and mammary tumor formation. *Stem Cells* *28*, 928-938.

Varelas, X., Samavarchi-Tehrani, P., Narimatsu, M., Weiss, A., Cockburn, K., Larsen, B. G., Rossant, J., and Wrana, J. L. (2010). The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-beta-SMAD pathway. *Developmental cell* *19*, 831-844.

Veltmaat, J. M., Mailleux, A. A., Thiery, J. P., and Bellusci, S. (2003). Mouse embryonic mammaryogenesis as a model for the molecular regulation of pattern formation. *Differentiation; research in biological diversity* *71*, 1-17.

Viloria-Petit, A. M., David, L., Jia, J. Y., Erdemir, T., Bane, A. L., Pinnaduwa, D., Roncari, L., Narimatsu, M., Bose, R., Moffat, J., et al. (2009). A role for the TGFbeta-Par6 polarity pathway in breast cancer progression. *Proceedings of the National Academy of Sciences of the United States of America* *106*, 14028-14033.

Wagner, K. U., Krempler, A., Triplett, A. A., Qi, Y., George, N. M., Zhu, J., and Rui, H. (2004). Impaired alveologenesis and maintenance of secretory mammary epithelial cells in Jak2 conditional knockout mice. *Molecular and cellular biology* *24*, 5510-5520.

Wagner, K. U., Wall, R. J., St-Onge, L., Gruss, P., Wynshaw-Boris, A., Garrett, L., Li, M., Furth, P. A., and Hennighausen, L. (1997). Cre-mediated gene deletion in the mammary gland. *Nucleic acids research* *25*, 4323-4330.

Wang, Q., Hurd, T. W., and Margolis, B. (2004a). Tight junction protein Par6 interacts with an evolutionarily conserved region in the amino terminus of PALS1/stardust. *The Journal of biological chemistry* *279*, 30715-30721.

Wang, Y., Engels, I. H., Knee, D. A., Nasoff, M., Deveraux, Q. L., and Quon, K. C. (2004b). Synthetic lethal targeting of MYC by activation of the DR5 death receptor pathway. *Cancer cell* *5*, 501-512.

Wang, Y., and Nathans, J. (2007). Tissue/planar cell polarity in vertebrates: new insights and new questions. *Development* *134*, 647-658.

Wang, Y. A., Shen, K., Wang, Y., and Brooks, S. C. (2005). Retinoic acid signaling is required for proper morphogenesis of mammary gland. *Developmental dynamics : an official publication of the American Association of Anatomists* *234*, 892-899.

Wanzel, M., Herold, S., and Eilers, M. (2003). Transcriptional repression by Myc. *Trends in cell biology* 13, 146-150.

Watson, C. J., and Kreuzaler, P. A. (2011). Remodeling mechanisms of the mammary gland during involution. *The International journal of developmental biology* 55, 757-762.

Weaver, V. M., Lelievre, S., Lakins, J. N., Chrenek, M. A., Jones, J. C., Giancotti, F., Werb, Z., and Bissell, M. J. (2002). beta4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. *Cancer cell* 2, 205-216.

Wei, M., Grushko, T. A., Dignam, J., Hagos, F., Nanda, R., Sveen, L., Xu, J., Fackenthal, J., Tretiakova, M., Das, S., and Olopade, O. I. (2005). BRCA1 promoter methylation in sporadic breast cancer is associated with reduced BRCA1 copy number and chromosome 17 aneusomy. *Cancer research* 65, 10692-10699.

Wei, M. C., Zong, W. X., Cheng, E. H., Lindsten, T., Panoutsakopoulou, V., Ross, A. J., Roth, K. A., MacGregor, G. R., Thompson, C. B., and Korsmeyer, S. J. (2001). Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science* 292, 727-730.

Welm, B. E., Dijkgraaf, G. J., Bledau, A. S., Welm, A. L., and Werb, Z. (2008). Lentiviral transduction of mammary stem cells for analysis of gene function during development and cancer. *Cell stem cell* 2, 90-102.

Westermarck, U. K., Wilhelm, M., Frenzel, A., and Henriksson, M. A. (2011). The MYCN oncogene and differentiation in neuroblastoma. *Seminars in cancer biology* 21, 256-266.

Wilson, A., Murphy, M. J., Oskarsson, T., Kaloulis, K., Bettess, M. D., Oser, G. M., Pasche, A. C., Knabenhans, C., Macdonald, H. R., and Trumpp, A. (2004). c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes & development* 18, 2747-2763.

Wingo, S. N., Gallardo, T. D., Akbay, E. A., Liang, M. C., Contreras, C. M., Boren, T., Shimamura, T., Miller, D. S., Sharpless, N. E., Bardeesy, N., *et al.* (2009). Somatic LKB1 mutations promote cervical cancer progression. *PloS one* 4, e5137.

Wiseman, B. S., Sternlicht, M. D., Lund, L. R., Alexander, C. M., Mott, J., Bissell, M. J., Soloway, P., Itohara, S., and Werb, Z. (2003). Site-specific inductive and inhibitory activities of MMP-2 and MMP-3 orchestrate mammary gland branching morphogenesis. *The Journal of cell biology* 162, 1123-1133.

Wodarz, A., Hinz, U., Engelbert, M., and Knust, E. (1995). Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell* 82, 67-76.

Wood, M. A., McMahon, S. B., and Cole, M. D. (2000). An ATPase/helicase complex is an essential cofactor for oncogenic transformation by c-Myc. *Molecular cell* 5, 321-330.

Woods, A., Johnstone, S. R., Dickerson, K., Leiper, F. C., Fryer, L. G., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M., and Carling, D. (2003). LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Current biology : CB* 13, 2004-2008.

Woods, D. F., and Bryant, P. J. (1991). The discs-large tumor suppressor gene of *Drosophila* encodes a guanylate kinase homolog localized at septate junctions. *Cell* 66, 451-464.

Wrobel, C. N., Debnath, J., Lin, E., Beausoleil, S., Roussel, M. F., and Brugge, J. S. (2004). Autocrine CSF-1R activation promotes Src-dependent disruption of mammary epithelial architecture. *The Journal of cell biology* 165, 263-273.

Wu, S., Cetinkaya, C., Munoz-Alonso, M. J., von der Lehr, N., Bahram, F., Beuger, V., Eilers, M., Leon, J., and Larsson, L. G. (2003a). Myc represses differentiation-induced p21CIP1 expression via Miz-1-dependent interaction with the p21 core promoter. *Oncogene* 22, 351-360.

Wu, S., Huang, J., Dong, J., and Pan, D. (2003b). hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts. *Cell* 114, 445-456.

Wysolmerski, J. J., Philbrick, W. M., Dunbar, M. E., Lanske, B., Kronenberg, H., and Broadus, A. E. (1998). Rescue of the parathyroid hormone-related protein knockout mouse demonstrates that parathyroid hormone-related protein is essential for mammary gland development. *Development* 125, 1285-1294.

Xing, P., Li, J. G., Jin, F., Zhao, T. T., Liu, Q., Dong, H. T., and Wei, X. L. (2011). Clinical and biological significance of hepsin overexpression in breast cancer. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research* 59, 803-810.

Yamashita, Y., Saito, Y., Murata-Kamiya, N., and Hatakeyama, M. (2011). Polarity-regulating kinase partitioning-defective 1b (PAR1b) phosphorylates guanine nucleotide exchange factor H1 (GEF-H1) to regulate RhoA-dependent actin cytoskeletal reorganization. *The Journal of biological chemistry* 286, 44576-44584.

Yamaji, D., Na, R., Feuermann, Y., Pechhold, S., Chen, W., Robinson, G. W., and Hennighausen, L. (2009). Development of mammary luminal progenitor cells is controlled by the transcription factor STAT5A. *Genes & development* 23, 2382-2387.

Yamanaka, T., Horikoshi, Y., Sugiyama, Y., Ishiyama, C., Suzuki, A., Hirose, T., Iwamatsu, A., Shinohara, A., and Ohno, S. (2003). Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. *Current biology : CB* 13, 734-743.

Yamanaka, T., Horikoshi, Y., Suzuki, A., Sugiyama, Y., Kitamura, K., Maniwa, R., Nagai, Y., Yamashita, A., Hirose, T., Ishikawa, H., and Ohno, S. (2001). PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex. *Genes to cells : devoted to molecular & cellular mechanisms* 6, 721-731.

Yang, W., Shen, J., Wu, M., Arsura, M., FitzGerald, M., Suldan, Z., Kim, D. W., Hofmann, C. S., Pianetti, S., Romieu-Mourez, R., *et al.* (2001). Repression of transcription of the p27(Kip1) cyclin-dependent kinase inhibitor gene by c-Myc. *Oncogene* 20, 1688-1702.

Yant, J., Buluwela, L., Niranjan, B., Gusterson, B., and Kamalati, T. (1998). In vivo effects of hepatocyte growth factor/scatter factor on mouse mammary gland development. *Experimental cell research* 241, 476-481.

Yeh, P. Y., Lu, Y. S., Ou, D. L., and Cheng, A. L. (2011). IkbpaB kinases increase Myc protein stability and enhance progression of breast cancer cells. *Molecular cancer* 10, 53.

Ylikorkala, A., Rossi, D. J., Korsisaari, N., Luukko, K., Alitalo, K., Henkemeyer, M., and Makela, T. P. (2001). Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice. *Science* 293, 1323-1326.

Yoshihara, K., Ikenouchi, J., Izumi, Y., Akashi, M., Tsukita, S., and Furuse, M. (2011). Phosphorylation state regulates the localization of Scribble at adherens junctions and its association with E-cadherin-catenin complexes. *Experimental cell research* 317, 413-422.

Yu, W., Datta, A., Leroy, P., O'Brien, L. E., Mak, G., Jou, T. S., Matlin, K. S., Mostov, K. E., and Zegers, M. M. (2005). Beta1-integrin orients epithelial polarity via Rac1 and laminin. *Molecular biology of the cell* 16, 433-445.

- Yu, W., Shewan, A. M., Brakeman, P., Eastburn, D. J., Datta, A., Bryant, D. M., Fan, Q. W., Weiss, W. A., Zegers, M. M., and Mostov, K. E. (2008). Involvement of RhoA, ROCK I and myosin II in inverted orientation of epithelial polarity. *EMBO reports* 9, 923-929.
- Yurchenco, P. D. (2011). Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harbor perspectives in biology* 3.
- Zagorska, A., Deak, M., Campbell, D. G., Banerjee, S., Hirano, M., Aizawa, S., Prescott, A. R., and Alessi, D. R. (2010). New roles for the LKB1-NUAK pathway in controlling myosin phosphatase complexes and cell adhesion. *Science signaling* 3, ra25.
- Zeller, K. I., Jegga, A. G., Aronow, B. J., O'Donnell, K. A., and Dang, C. V. (2003). An integrated database of genes responsive to the Myc oncogenic transcription factor: identification of direct genomic targets. *Genome biology* 4, R69.
- Zeller, K. I., Zhao, X., Lee, C. W., Chiu, K. P., Yao, F., Yustein, J. T., Ooi, H. S., Orlov, Y. L., Shahab, A., Yong, H. C., *et al.* (2006). Global mapping of c-Myc binding sites and target gene networks in human B cells. *Proceedings of the National Academy of Sciences of the United States of America* 103, 17834-17839.
- Zen, K., Yasui, K., Gen, Y., Dohi, O., Wakabayashi, N., Mitsufuji, S., Itoh, Y., Zen, Y., Nakanuma, Y., Taniwaki, M., *et al.* (2009). Defective expression of polarity protein PAR-3 gene (PARD3) in esophageal squamous cell carcinoma. *Oncogene* 28, 2910-2918.
- Zhan, L., Rosenberg, A., Bergami, K. C., Yu, M., Xuan, Z., Jaffe, A. B., Allred, C., and Muthuswamy, S. K. (2008). Deregulation of scribble promotes mammary tumorigenesis and reveals a role for cell polarity in carcinoma. *Cell* 135, 865-878.
- Zhang, L., Li, J., Young, L. H., and Caplan, M. J. (2006). AMP-activated protein kinase regulates the assembly of epithelial tight junctions. *Proceedings of the National Academy of Sciences of the United States of America* 103, 17272-17277.
- Zhang, X., Farrell, A. S., Daniel, C. J., Arnold, H., Scanlan, C., Laraway, B. J., Janghorban, M., Lum, L., Chen, D., Troxell, M., and Sears, R. (2011). Mechanistic insight into Myc stabilization in breast cancer involving aberrant Axin1 expression. *Proceedings of the National Academy of Sciences of the United States of America*.
- Zhang, X., Wang, P., Gangar, A., Zhang, J., Brennwald, P., TerBush, D., and Guo, W. (2005). Lethal giant larvae proteins interact with the exocyst complex and are involved in polarized exocytosis. *The Journal of cell biology* 170, 273-283.
- Zhao, B., Li, L., Wang, L., Wang, C. Y., Yu, J., and Guan, K. L. (2012). Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis. *Genes & development* 26, 54-68.
- Zheng, B., and Cantley, L. C. (2007). Regulation of epithelial tight junction assembly and disassembly by AMP-activated protein kinase. *Proceedings of the National Academy of Sciences of the United States of America* 104, 819-822.
- Zheng, B., Jeong, J. H., Asara, J. M., Yuan, Y. Y., Granter, S. R., Chin, L., and Cantley, L. C. (2009). Oncogenic B-Raf negatively regulates the tumor suppressor LKB1 to promote melanoma cell proliferation. *Molecular cell* 33, 237-247.
- Zhong, D., Liu, X., Khuri, F. R., Sun, S. Y., Vertino, P. M., and Zhou, W. (2008). LKB1 is necessary for Akt-mediated phosphorylation of proapoptotic proteins. *Cancer research* 68, 7270-7277.
- Zhou, Z., Wang, J., Cao, R., Morita, H., Soininen, R., Chan, K. M., Liu, B., Cao, Y., and Tryggvason, K. (2004). Impaired angiogenesis, delayed wound healing and retarded tumor growth in perlecan heparan sulfate-deficient mice. *Cancer research* 64, 4699-4702.
- Zhu, Z., Jiang, W., and Thompson, H. J. (2003). Mechanisms by which energy restriction inhibits rat mammary carcinogenesis: in vivo effects of corticosterone on cell cycle machinery in mammary carcinomas. *Carcinogenesis* 24, 1225-1231.
- Zhurinsky, J., Shtutman, M., and Ben-Ze'ev, A. (2000). Differential mechanisms of LEF/TCF family-dependent transcriptional activation by beta-catenin and plakoglobin. *Molecular and cellular biology* 20, 4238-4252.
- Zindy, F., Eischen, C. M., Randle, D. H., Kamijo, T., Cleveland, J. L., Sherr, C. J., and Roussel, M. F. (1998). Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes & development* 12, 2424-2433.